AN INTRODUCTION

TO

PRACTICAL HISTOLOGY

BY

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PREFACE.

DURING the last few years several valuable manuals of Practical Histology have appeared in this country, both as original works and translations. The important treatise by M. Ranvier ("Traité technique d'Histologie"), now passing through the press in Paris, will also, it is hoped, soon be added to the list. The present work makes no pretensions to supersede any of these its predecessors. Indeed, neither this nor any other work yet published can profess to contain everything that the student of practical histology ought to know.

In the course of our own investigations we have had occasion to give special attention to some of the chemical agents and dyes in common use, and also to examine the scientific journals, especially those of Germany and France, for records of new modes of research. To present the information so acquired in a convenient form is one of the objects of this book. As instances of the methods derived from foreign

sources may be mentioned that of Von Ebner for the examination of bone; of Gerlach for studying the termination of nerve fibrils in muscle; of Cornil for tracing the course of amyloid degeneration; of Schwalbe for elastic fibres; of Löwit in the combined application of gold and formic acid in the study of nerves; and of Ranvier in the use of purpurine. These are a few examples of histological methods which have been described so recently that they are scarcely yet known to English readers. We trust that the abstracts we have given of these and other methods may be useful even to histologists of long experience.

Our primary object, however, being to make the work useful to those who are only beginning to use the microscope, we have arranged it in the form of a manual of Practical Histology. The plan we have adopted is to exemplify the various methods by explaining in considerable detail their application to organs which are specially suited for this purpose.

In carrying out this idea we have been unavoidably led to treat at considerable length the histology of some of the tissues, and in doing so have had occasion in several instances, as, for example, when studying the structure of muscle, cartilage, and the retina, to explain views that diverge widely from those taught in the text-books. In doing so we have, however, been careful to give full details respecting the methods we have employed. In regard to the points on which there is much difference of opinion, the student will find it

of greater advantage to be able to examine them for himself, even without arriving at a definite conclusion, than to follow implicitly the teaching of any authority whatsoever.

It follows from this plan that the details of the mode of application of any one reagent are sometimes found in different parts of the book, but any inconvenience which might arise from this arrangement will be obviated by consulting the index.

Amongst the works which have been useful to us in the execution of our task we have to express our obligations more especially to the published part of Ranvier's treatise, and to Exner's "Leitfaden bei der Mikroskopischen Untersuchung Thierischer Gewebe." The section on embryology has been translated literally from the latter book.

LONDON, *May*, 1877.

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In ordinary histological work the following **instruments** are indispensable:

Two **needles** fixed in handles, or two needle-holders into which needles can be fitted.

Strong and fine **scissors.**

A **scalpel.**

A **razor,** flat on the under surface and slightly grooved on the upper. Instead of a razor a **knife** specially fitted for cutting sections may be used. Those sold in London and Edinburgh are constructed after the model of a Vienna maker. The knife on account of its weight and length has some advantages, but a good razor is sufficient for most purposes.

A **section-lifter** or **spud** for removing sections from fluids. One can be readily made by beating flat the end of a piece of copper wire, and bending the flattened part.

Several **glass rods.**

Pipettes (which are made by heating a glass tube and drawing out its extremities to points).

Small **phials** containing glycerine, acetic acid, distilled water, dammar varnish or Canada balsam. It is convenient to have the cork perforated by a glass rod or tube.

A group of phials fixed in a **glass tray,** into which a perforated piece of cork is fitted, is used in Paris for this purpose,

and will be found very convenient. Mr. Hawksley, instrument-maker, Oxford Street, London, can supply this tray.

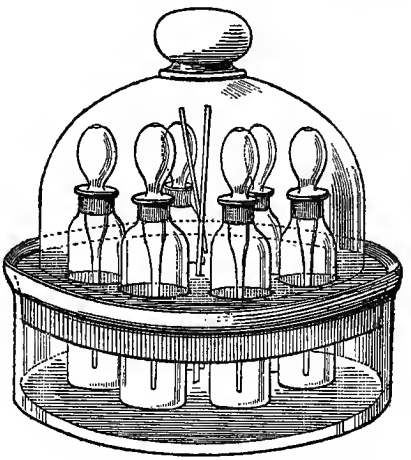

Fig. 1. Tray for Re-agent Phials.

Brunswick black or **asphalt**, for cementing preparations. We prefer Brunswick black, which can be had best at the ordinary oil-shops. (**Sealing-wax** dissolved in spirit can also be used. For certain purposes preparations are cemented by melted **paraffin**.)

Glass slides, three inches by one inch, and thin **cover-glasses**, three-quarter inch square.

A **German hone**, eight inches long and two inches wide, for sharpening the section-knife or razor.

To make useful sections the razor must be kept very sharp, and every worker should know how to sharpen it for himself. If possible this should be learned from a cutler. The following hints may however be useful. The grooved surface of the razor is laid flat on the well-oiled stone, and is carried steadily backwards and forwards. On account of the groove the pressure rests largely on the edge, which is therefore rapidly ground. The razor is then turned and the flat surface laid on the hone. It is evident that,

presuming the knife to be quite flat, a movement like that applied to the other side would in this case only wear down the whole side of the knife. It is necessary therefore to raise the back part of the razor very slightly from the hone, so that the pressure may be applied to the edge. But for the particular kind of edge which is needed, it is important that the knife should be raised to the slightest possible degree. The knife is in this case not rubbed backwards and forwards, but carried always with the edge against the stone, and it is important that the angle formed by the blade and the surface should be kept uniform. When a good edge has been formed, it should be perfected by being passed several times over one of the leather straps sold for the purpose.

A few **camel's hair brushes, watch-glasses,** and small **porcelain capsules** are also required.

The **microscope** used should be provided with at least two objectives, one magnifying 50 to 80, and another 300 to 400 diameters. Other powers are convenient, especially one of about 150 diameters. For some kinds of research a good immersion lens, magnifying 700 to 1000 diameters, is useful. But with the two objectives first mentioned histology may for a time be well studied.

There is indeed very little that may not be done with a power of 300 to 400 diameters, and probably better done so than with a power of 1000 diameters. The use of high powers is as a rule to be avoided.

The assistance of an experienced microscopist should be obtained in the selection of the objectives. For those who are accustomed to microscopic work, the molecular movements in the corpuscles which are always present in saliva form a good test object.

In using the microscope direct sunlight and artificial light should be avoided when possible. The best light is obtained from white clouds in an otherwise clear sky. Artificial light may be tempered by being made to pass through tinted glass or water. Those who work with artificial light find logwood staining less trying to the eyes than carmine. But whilst coloured elements and strongly-marked outlines are readily

seen, it is doubtful whether microscopic studies can be carried on very favourably by artificial light.

The magnifying power should not be increased by the use of a high ocular. The gain is in this case usually more than counterbalanced by loss of definition. The lowest ocular should be employed with immersion lenses of high power.

The magnifying power is increased by drawing out the tube of the microscope.

The method of **examining simple objects** may be practised by looking at milk. A drop is placed on the slide, and a cover glass held by forceps is brought over it in the following manner. One border rests on the slide, and the other on the lower blade of the forceps, which is gradually lowered until the glass first touches the fluid and finally is quite level.

The cover-glass should never be allowed to *fall* on the fluid. By attending to these precautions air bubbles are excluded. The drop should be sufficiently large to extend to the edges of the cover-glass, but not more so.

The slide being then placed on the stage the mirror is moved until the light is reflected to the object which should be over the centre of the stage-aperture. The tube of the microscope, armed with a power of 300 to 400 diameters, is moved downwards until it is near the cover-glass. The observer looking through the microscope, moves the tube slowly farther downwards until an indistinct image of the oil globules becomes visible. He then moves the screw that constitutes the fine adjustment very slowly until the objects are focused. By alternately raising and depressing the objective to a very slight extent, he finds that the appearance of the globules varies according as the upper surface or their centre is in focus. This carrying of the focus upwards and downwards through an object is indispensable in every kind of histological investigation, especially when high powers are used.

Beginners must avoid bringing the objective into contact with the cover-glass. If they do so, they may injure their instrument as well as spoil their preparation.

Low power objectives are not brought so near the cover-glass. Facility in their use is soon acquired by examining such objects as a hair or a fly's wing simply inclosed between the glasses.

An immersion lens is used by placing a very small drop of distilled water on the surface of the objective. It is then brought down cautiously until the water comes in contact with the cover-glass, further movement being regulated by the fine adjustment. After use it should be carefully and gently dried.

For the **measurement** of objects under the microscope a micrometer eye-piece and a stage micrometer are necessary. The former is an ocular containing in the focus of the upper lens a piece of glass on which a scale has been marked. When an object is to be measured, this eye-piece is placed in the microscope, the object brought into focus, and the number of divisions of the scale over which it extends is noted. The stage micrometer is a glass slide, on which a very fine scale marking known fractions of a millimeter or inch is engraved. This stage micrometer is inserted instead of the object which is to be measured (the tube of the microscope being kept in the same position), and an observation is made of the number of divisions covered by the part of the eye-piece scale which is equal to the length of the object to be measured. The number is the length required. As the same objective with the same position of the tube will always magnify with the ocular micrometer to the same degree, by keeping the conditions the same the use of the stage micrometer may be afterwards dispensed with. The value of a set of lenses in a given position of the tube may be thus determined once for all.

Tissues are examined fresh in serum or in ½ per cent. salt solution;[1] or they are hardened and thin sections are ex-

[1] The strength of solutions is expressed in percentages for the sake of brevity. Instead of the phrase 1 *part of salt in* 200 *water*, we shall in this work say ½ *per cent. salt solution.* These solutions are most conveniently prepared by using the decimal system of weights and measures; 1 gramme of salt dissolved in 200 cubic centimeters distilled water giving, for example, the ½ per cent. solution required.

amined. They are also disintegrated by needles either fresh or after being hardened until parts sufficiently transparent are obtained. In either case they may be dyed by staining agents, various elements having special elective affinities for the dyes. Some agents both stain and harden.

The following are the **hardening agents** in common use :

Absolute alcohol, methylated alcohol (or simply **alcohol**), and alcohol diluted with two parts water, which we shall henceforth term **diluted alcohol.** These different strengths have different effects.

Solutions of **chromic acid** in various degrees of dilution. The strength generally used is from $\frac{1}{8}$th to 1 per cent. A standard solution of 2 per cent. to be diluted as required is useful.

Bichromate of potash in solution of 1 to 2 per cent. **Bichromate of ammonia** has a similar action. **Muller's fluid** is composed of a 2 per cent. solution of bichromate of potash, with the addition of sulphate of soda in the proportion of 1 per cent. A short action of the bichromate or of diluted alcohol (24 to 48 hours) fits tissues for being teased out, and is specially adapted for isolation of epithelium.

Picric acid in saturated solution hardens tissues sufficiently for cutting.

Chloride of Palladium is recommended by F. E. Schultze for staining and hardening some tissues. It is used in $\frac{1}{10}$th per cent. solution, a few drops of hydrochloric acid being added.

Hardening in gum has been recommended by Brücke. The part to be cut after being 1 to 2 days in chromic acid, or Muller's fluid, is placed in a solution of gum arabic of the consistence of syrup for 24 hours. It is then hardened in alcohol and cut. The sections are freed from the gum which is in their interstices by being placed in water for 24 hours.

Weak solutions of chromic acid, and of bichromate of

potash and diluted alcohol have a macerating effect on tissues during the first 24 hours.

It is important in hardening tissues with chromic acid, bichromate of potash, and picric acid to place only a small portion of tissue in a relatively large quantity of fluid, and to change the latter after 24 or 48 hours.

As a general rule it is convenient to harden in chromic acid or Müller's fluid for a few days, and then to complete the process by 24 hours of methylated or absolute alcohol, although there are some tissues for which this rule must be modified.

Some histologists harden in a mixture of chromic acid solution and alcohol.

Potash and **Soda** are used in weak and in strong solutions, the strong solutions of potash sometimes preserving tissues that are destroyed by weaker ones.

We have found special results by using a solution of caustic potash in an equal weight of distilled water. Necessary conditions of success are chemical purity of the potash, its perfect dryness, and a certain temperature. When not less than 15 grammes is used the temperature should rise to 130° or 140° Fahr. When it falls to 107° the fresh tissues are placed in the solution, and after a few minutes are broken up and examined in a drop of it. (See further under *Cornea.*) In referring to this method we shall use the term **saturated potash solution.**

The indications for the use of the **mineral acids** and **acetic acid** will be found further on.

To procure a medium for the examination of tissues which produces no chemical change in them, the aqueous and **vitreous humours** and **blood serum** are available. In the frog the aqueous humour is best obtained by opening the anterior chamber from the edge of the cornea with a cataract knife immediately over a slide. A large drop may be obtained on the blade of the knife. To obtain blood serum cut the frog's head off and let the blood from the trunk drop into a small capsule or watch-glass. The muscular contractions produced by

passing a needle into the spinal column hasten its flow. The blood is covered and allowed to remain undisturbed for an hour or two, by which time the serum is available for use. By adding a few drops of carbolic acid solution it may be preserved indefinitely as **carbolised serum.**

For mammalian tissues the aqueous humour of the ox eye is very convenient. The fresh eye is held over a small vessel, the cornea downwards and within it. A sharp scalpel is then drawn across and through the centre of the cornea and the aqueous humour falls into the vessel. A large quantity of fluid can be obtained from the vitreous humour of the ox eye. It is convenient to break it up with scissors and needles to free the fluid from the membranous parts.

Blood serum and aqueous humour have valuable macerating qualities whilst they at the same time preserve the most delicate elements. Putrefaction may be prevented by addition of a few drops of strong solution of carbolic acid—in very feeble proportion to the quantity of fluid.

Iodised serum is now much used for maceration and preservation of delicate tissues. It is prepared by collecting amniotic fluid in a shallow vessel or bottle containing iodine. The vessel must be shaken or the bottle turned over daily.

Ranvier recommends the following method: The serum is mixed with tincture of iodine in large proportion. Filter, and add a little of this strongly iodised serum daily to the ordinary serum, which is thus prevented from putrefying. In one or two months a very strongly iodised serum is obtained which is available to iodise fresh serum.

Amniotic fluid is not easily obtainable in this country. We have used in its stead **iodised vitreous humour** with excellent results. The vitreous humour of four or six ox eyes is collected in a small vessel and thoroughly broken down by forceps and scissors. It is then poured into a wide bottle and a little iodine added to it. If shaken once or twice during the first few days the iodine soon begins to darken the fluid, and the solution once begun goes on rapidly, so that after a time the

vitreous humour becomes strongly iodised. This strongly iodised serum is filtered and may then be used to iodise to a slighter degree vitreous humour from fresh eyes. It is convenient to have samples strongly and weakly iodised.

A small portion of fresh tissue is placed in a weakly iodised serum until sufficiently macerated to be teased out, fresh serum being added as the liquor becomes colourless by the absorption of the iodine. This is repeated until the fluid remains brown.

Osmic acid not only stains but hardens tissues sufficiently for section-cutting, and when necessary it is possible to dispense with the subsequent action of alcohol. The skin and cornea can also be cut directly after the reduction of **chloride of gold** in acidulated water.

The most commonly used **staining agents** are carmine, hæmatoxylin, and the picrocarminate of ammonia, which combines the action of pikric acid and carmine. For some purposes the aniline dyes possess special advantages, and are being more and more used.

The **carmine** of commerce varies, and good carmine is not easily obtained. The best is to be had in Paris. It is insoluble in water until ammonia has been added. As ammonia acts destructively on the tissues, it is allowed to evaporate before the solution is ready for use. A number of formulæ are given. The following is from Frey: Several grains of carmine are added to about an ounce of water containing a few drops of ammonia. Part of the carmine dissolves. The mixture is filtered and the carmine in solution passes through. If it still smells of ammonia the bottle should be left unstoppered for 12 to 24 hours. The carmine which remains undissolved on the filter can be used further.

Ranvier gives the following proportions: Distilled water, 100; ammonia, 1; carmine, 1. Rub the carmine with a little water, pour on the ammonia, and then add the water. Drive off the excess of ammonia by a water bath until the carmine begins to deposit.

Carmine solutions like the above do not keep well.

By the addition of glycerine and alcohol the solution can be kept indefinitely, but the presence of these ingredients should not be forgotten. In some special instances they have advantages, but for many purposes the simple ammoniacal solution is preferable.

To avoid the effects of ammonia on the tissues Schweigger-Seidel introduced an **acid carmine**. He neutralised the ammonia with acetic acid and filtered the solution. Preparations stained in this solution are afterwards placed for a short period in ½ per cent. hydrochloric acid. The effect of acids is to remove all diffuse colouring and to bring the nuclei into especial prominence.

Carmine preparations, when not preserved in Canada balsam or dammar, should be mounted in glycerine faintly acidulated with acetic acid. Ranvier recommends as preferable one part of formic acid in 100 parts of glycerine. (Carmine was first used by Gerlach.)

The **picrocarminate of ammonia** (Ranvier) acts neither as acid nor alkali. It is prepared by adding a solution of carmine in ammonia to a saturated solution of picric acid. When by evaporation over a water bath the fluid has been reduced to a fifth of its previous volume it is allowed to cool and filtered. As the filtered fluid evaporates the solid picrocarminate is obtained. It should dissolve completely in distilled water. M. Ranvier recommends a 1 per cent solution.

Hæmatoxylin solution (Bœhmer) is thus prepared: Dissolve 20 grains of hæmatoxylin in ½ an ounce of absolute alcohol, and 2 grains of alum in 1 ounce of water. Some drops of the first solution are added to the second, which in a short while becomes a beautiful violet. It improves after being kept a few days, and should be filtered before use. It should always be prepared some days before being required, and should be exposed to the action of sun light.

The same effect is obtained by using a **solution of logwood.** Several formulæ, not varying much from each other, have been

given for the preparation of this dye. They are all good provided the extract of logwood used is good. And this is practically a difficulty. The *extractum hæmatoxyli* sold by many druggists does not yield the dye required for histological purposes. We have been informed by competent authority that when this is the case the fault is in the preparation of the extract.

In default of a good extract Bœhmer's solution of hæmatoxylin will be found equally useful. It has the advantage of being certain, and is easily prepared.*

Logwood solution may be prepared by rubbing to powder in a mortar 10 grammes each of extract of logwood and alum, and then adding gradually 100 CC of water, stirring whilst the water is being added. After some hours the liquor is filtered into a stoppered bottle, and is exposed to sunlight. If good it should stain almost at once, but it improves after several days. A few drops of this strong solution can be added to a watch-glassful of water when required, the diluted fluid being then passed through filter-paper. It is also useful for colouring glycerine, a few drops sufficing for a considerable quantity of glycerine.

We have recommended a combination of the use of **logwood and acetic acid**, which has often given us excellent results. A portion of tissue is rapidly stained through and through with a concentrated solution. It is then mounted in glycerine, and diluted acetic acid is drawn under the cover-glass with blotting-paper. The tissue rapidly bleaches, but the nuclei do not become bleached so rapidly as the other elements. As soon as they become visible the acetic acid is replaced as quickly as possible by glycerine or water in order to arrest the decoloration. The number of nuclei stained in this way sometimes exceeds that seen by any other process known to us.

* Mr. Martindale, chemist, New Cavendish Street, London, bestows especial care on the preparation of logwood and picrocarminate solutions.

Foster and Balfour give the following as Kleinenburg's **alcoholic solution of hæmatoxylin**: (1.) Make a saturated solution of crystallised calcium chloride in 70 per cent. alcohol, and add alum to saturation. (2.) Make also a saturated solution of alum in 70 per cent. alcohol. Add (1) to (2) in the proportion of 1 to 8. To the mixture add a few drops of a saturated solution of hæmatoxylin in absolute alcohol. Tissues can be hardened and stained at once by this solution. Sections stained in it do not require to be brought in contact with water.

Quinoléine blue is dissolved in rectified spirit, and an equal part of water is afterwards added. It is a powerful dye, and is greatly diluted for use. The staining has a distinctive shade in different elements of the tissues. It has an especial affinity for fat, which it stains a deep blue. The effect is not permanent. (Ranvier.)

Molybdate of ammonia, recommended by Krause, and, as appears from his work on "General Anatomy," frequently and successfully employed by him, is used in 5 per cent. solution. It stains blue. The colour is deepened by subsequent maceration in gallic or pryogallic acid.

Of the **aniline colours** there are used:

Aniline blue, soluble in alcohol and insoluble in water, and **aniline blue**, soluble in water.

Magenta (known also as **roseine**, and in a somewhat modified state as **fuchsine**) is the **acetate of rosaniline**. It is soluble in water and more so with the addition of a little alcohol.

A dye sold in England as **aniline red**, of which we have not been able to find the exact composition, sparingly soluble in water, but freely when a little alcohol is added, we have found very useful in staining tissues which had been previously treated by osmic acid.

Eosin, first proposed by Fischer in 1875 (Schultze's "Archiv.," vol. xii.), is a valuable dye. It dissolves both in water and alcohol, a few drops of a solution of 1 in 10 to 20

of water being added to a watch-glassful of the water or alcohol as required. It stains epithelium a dark red, the axis cylinder of nerves, but not the medulla, colours also the areolar tissue, the nuclei of ganglion cells and their processes, the latter feebly. The amyloid substance in that form of degeneration stains bright red. Fresh tissues placed in an alcoholic solution are hardened and stained simultaneously.

Dr. Wissozky of Kasan (Schultze's "Archiv.," vol. xiii.) states that it is specially valuable for the recognition of blood corpuscles. It stains the hæmoglobin. He recommends a solution of eosin, 1 ; alum, 1 ; alcohol, 200.

The **violet of methylaniline**, lately recommended by M. Cornil, is decomposed into two colours in certain tissues, each of the colours having a special election for different elements. We translate the following remarks regarding it from the "Compte rendu des Séances de la Société de Biologie," Paris, 1875, p. 200. "M. Cornil had employed, as a colouring matter, in microscopic preparations, two kinds of violet of methylaniline; one, violet of methylaniline pure, the other called violet of Paris, also obtained in crystals and somewhat bluer than the former. The latter bears the trade mark **350 N.**; and gives the best preparations. Both of them come from the manufactory of M. Poirier. A watery solution, considerably diluted, is placed in contact with the objects that are to be examined, whether teased out by needles or cut in thin sections. It is better to employ a weak than a strong solution, as the imbibition is then more regular. It is necessary, therefore, to employ a rather large quantity of this filtered solution. After four or five minutes the staining is as deep as possible. Preparations either hardened in pure alcohol, or by Müller's fluid, gum and alcohol, are easily stained after thin sections have been placed for some minutes in water. The stained sections are preserved in water, or in water to which glycerine has been added. If the glycerine is acid, or if a very small proportion of acetic acid is added to it, part of the colouring matter is dissolved, and the glycerine surrounding the preparation is

coloured a very faint violet. But the nuclei of cells are then seen all the better. Alcohol, the essences of turpentine and cloves, dissipate the violet and make the elements colourless.

"The interest and utility of the employment of this colouring substance consist in this, that in impregnating certain tissues it is decomposed into two colours, one a red violet, the other a blue violet, which act, each of them, with great precision on different elements of the tissue. This perfectly constant election of the red for certain elements, of the blue for others, renders this substance precious for histological analysis. The dissociation of these two colours of the violet of methylaniline had been tried by chemical means by M. Lauth, without success.

"It is effected, however, at once by tissues affected by amyloid degeneration and by cartilage. The red-violet fixes itself on colloid points of gelatinous appearance, and the blue-violet on fibres and cells."

M. Cornil has shown that the use of this dye surpasses all methods hitherto known for the recognition of elements affected by amyloid degeneration; those which are thus altered staining red-violet, whilst the surrounding unaffected elements are coloured blue-violet.

Purpurine, a dye extracted from madder, has been lately recommended by Ranvier, and we can bear testimony to its value. It is prepared for use thus: "A solution of 1 part alum in 200 distilled water is brought to the boiling point in a porcelain capsule, and a small quantity of solid purpurine rubbed up in a little distilled water is added to it. The purpurine is dissolved in a few minutes. There should remain a small quantity of purpurine undissolved, which indicates that the solution is concentrated. It is filtered whilst still hot into rectified spirit. The alcohol should constitute a fourth part of the total volume of the mixture. The fluid thus obtained is of a beautiful orange red in transmitted light; it is fluorescent. At the end of a month there is a slight precipitate. It can still be used after this has formed, but it has lost somewhat of its colouring

power. It is better then to use a freshly prepared solution." For information regarding its effects on various tissues the reader is referred to M. Ranvier's memoir in the "Archives de Physiologie" for 1874. Purpurine must not be confounded with a simple extract of madder. We can unfortunately give no information regarding its mode of preparation. A substance lately sold as purpurine in England yielded no results like those described by Ranvier. The sample we have used was brought from Paris, and we have been able to satisfy ourselves of its value in preparations of the omentum, cornea, and retina more especially. In using it the effect of the proportion of alum and alcohol the solution contains must be kept in mind. Tissues are allowed to remain in it 24 to 48 hours.

There is no doubt that before long genuine purpurine will be available for English histologists.

Aniline blue-black, recommended by Mr. Sankey for staining the nerve-cells of the brain and spinal cord, is prepared by dissolving 5 centigr. of the dye in 2 CC. water and adding it to 99 CC. methylated spirit. Filter.

Of these various dyes carmine, and still more haematoxylin, stain nuclei more especially. The aniline dyes may be said in a general way to stain all the elements, although as the nucleated part of the cell is thicker than the other parts, it appears more deeply stained. A double staining, as for example by aniline and hæmatoxylin, is thus sometimes useful. Purpurine, though not a powerful dye, has a peculiar affinity for nuclei, and for some purposes cannot at present be replaced by any other known to us.

The following metallic solutions are indispensable: A $\frac{1}{2}$ per cent. solution of **nitrate of silver**; a $\frac{1}{2}$ per cent. solution of **chloride of gold**. The gold chloride is sold in glass tubes which contain 1 gramme. For some special purposes a $1\frac{1}{2}$ per cent. solution has been lately recommended. These solutions can be diluted at will. One per cent. solution of **osmic acid**. A solution of this strength is sold, and when there is not much used it is convenient to buy it thus ready prepared. The acid

is sold in tubes containing one gramme. Although a 1 per cent. solution, and even a 2 per cent. solution are sometimes used, it will be found that for most purposes a $\frac{1}{2}$ per cent. solution is sufficiently strong. Sometimes weak solutions such as $\frac{1}{10}$th per cent. are employed. For general purposes $\frac{1}{2}$ per cent. and $\frac{1}{10}$th per cent. solutions may be kept ready for use.

All these solutions of osmic acid must be kept in well-stoppered bottles, which should be covered with thick non-transparent paper or fitted into leathern cases. When they are used care should be taken not to expose the contents to light. They are exceedingly liable to be altered by its action, but if sufficient precautions are taken they may be kept unchanged for many months.

The double salt, the **chloride of gold and potassium**, is recommended by Gerlach in very weak solution for the demonstration of nerve-fibrillæ.

Injection masses. The mass now commonly used is a mixture of soluble Prussian blue and gelatine. **Prussian blue** was first proposed for this purpose by Brücke. The following formula for preparing it is taken from Exner's book:

"A. Dissolve 217 grammes of potassic ferrocyanide in a litre of water.

"B. Take a litre of 10 per cent. solution of ferric chloride.

"Add to each of these solutions two litres of a saturated solution of sulphate of soda. Then add the ferric chloride solution to the ferrocyanide, stirring the while. The precipitate is filtered through new flannel and the first washings returned. This is repeated for several days until the solution passed through a filter is of a deep blue colour. It is then soluble. The mass is now pressed, broken into small pieces, and dried in the air. An aqueous solution of this mass can be injected cold, or it can be warmed to the temperature of the body."

Soluble Berlin blue can be purchased.

A saturated solution kept ready for use is convenient. When employed for injections it is usually mixed with a solution of gelatine, prepared thus: The gelatine, which must be of the

best quality, is soaked for an hour in water. The water which is not absorbed is then poured off and the gelatine melted over a water-bath. The solution of Prussian blue being heated to the same temperature, the solution of gelatine is added to it gradually, the mixture being stirred. It is filtered (still hot) through new flannel, and then kept at a temperature of 40° C. until it is injected.

A carmine mass is prepared with gelatine and an ammoniacal solution of carmine. It is essential that the carmine be neutralised, as if alkaline it becomes diffused through the tissues, and if acid the carmine is deposited. An ordinary ammoniacal carmine solution is prepared and filtered. Warm it and add to it $\frac{4}{5}$ths of the warm solution of gelatine, prepared as directed for the Prussian blue mass, constantly stirring. Add acetic acid to the remaining fifth of the gelatine solution, and then add this acid mixture drop by drop to the carminised solution, until the ammonia is completely neutralised. This is the delicate point of the operation. As the odour of ammonia becomes faint the acid gelatine must be added very cautiously, and as soon as it cannot be perceived no more acid is added. Assistance is also afforded by the colour. As long as there is any free ammonia the solution is of a cherry-red colour, but as soon as it is all neutralised the colour changes to a fiery red. If the solution when prepared shows a granular deposit under the microscope, too much acid has been added, and the mass is useless.

On account of the uncertain strength of solutions of ammonia it is impossible to give formulæ for the exact proportions for a carmine mass. As approximative quantities may be given: 1 dram of carmine; 1 dram liq. ammon. (·880); $\frac{1}{2}$ dram of acetic acid concent. (of commerce); 5 ounces gelatine solution; but in no case should any standard be trusted other than those of colour and smell. The quality of the gelatine, its careful purification by being passed through new flannel, the maintenance of the solutions and of the animal at a temperature of 40° C., and the exact neutralisation of the

ammonia, are the requisite conditions for a successful injection.

A 2 per cent. solution of Berlin blue is sometimes used for injecting bloodvessels, but it does not give so good results as the gelatine mixture. It is suitable for injecting the lymphatics by puncture.

A solution of alcannin in turpentine is recommended by Ludwig for injecting lymphatics.

Injections are made either by means of a syringe or by a special apparatus. For most purposes a syringe answers perfectly well, especially for bloodvessels and lymphatics, and practically it is the syringe that is generally used by the most successful injectors.

Syringes, specially made for injections, are sold by the instrument-makers. They are provided with several cannulas of various calibre, and a separate piece containing a stop-cock to connect the cannula and the syringe.

In selecting a fine cannula it is well to choose one with an oblique opening and a "collar" immediately behind it by which the ligature can be easily fixed. This "collar" is more convenient than the grooves which are often filed on the end of the instrument.

Glass cannulas can be easily extemporised for injection with metallic solutions.

The insertion of the cannula into a small vessel is often a matter of considerable difficulty.

It is convenient to stretch the vessel by a ligature which is attached to it as soon as it has been laid bare. It is then easier to cut a small piece out of the wall of the vessel with sharp scissors. This opening is slightly extended and the cannula inserted. It is then firmly fixed near its point with a ligature, which is further fastened tightly at the wider end. Before the stop-cock piece is fitted into the cannula the latter is filled with salt solution drop by drop in order to expel the air which it contains. If the air is not all expelled it is driven onwards with the injection mass and produces a bad result.

The stop-cock piece is then fitted into the cannula and filled with injection mass in the same way. The syringe charged with the mass is next inserted into it, and the injection is proceeded with. The object of first filling the cannula with salt solution is also to prevent its being obstructed by a mixture of blood and injection mass.

Small animals can be most easily injected from the aorta. The creature is killed by chloroform, and immediately placed in water at 38° to 40° C. The thorax is laid open, the cannula inserted in the aorta, and an opening made in the right ventricle. The mass is driven on slowly and steadily. First blood and then the fluid which is being injected passes out of the right ventricle. As soon as the injection mass passes unmixed with blood the opening in the right ventricle is secured, and any cut vessel from which the fluid is escaping secured by clips. The pressure is then kept up until the distended eyeball and the colouration of the tongue or nose and lips shows that the injection has penetrated sufficiently. The stop-cock is then turned on to prevent regurgitation of the fluid, the syringe removed, the cannula of course retained, and the animal put at once in equal parts of alcohol and water, and allowed to remain in it for some hours, so that the gelatine may become solid. If a carmine mass has been used alcohol and water is the only fluid suitable for hardening, and a few drops of acetic acid should be added to prevent the carmine becoming diffused when in contact with the tissues. If Prussian blue has been injected, either alcohol, Müller's fluid, or picric acid may be used.

If an organ instead of the entire animal is to be injected, the same principles of operating must be adhered to.

The art of injecting can only be acquired by practice. The chief points to be borne in mind are the temperature of the mass and the animal, the purity of the former, and the exclusion of air in beginning to push the injection. Any granular precipitate in the mass must be specially guarded against.

In order to obtain a constant and known pressure, various

forms of apparatus have been invented; the principle on which they are constructed being mostly the same in all of them. The mass to be injected is placed in a bottle fitted with an air-tight caoutchouc stopper. The stopper is pierced by two glass tubes, one reaching nearly to the bottom at one end, and being bent and connected with a caoutchouc tube armed with a cannula at the other. The other tube does not extend far into the bottle, and is connected by means of a caoutchouc tube with another bottle which contains air. The air in this second bottle can be compressed in various ways, the amount of compression regulating the force with which the injection fluid is driven from the first bottle.

One of the simplest of these apparatus is described and figured by Ranvier ("Traité technique," p. 128). It consists of a straight support on which two vessels connected by an india-rubber tube are fitted. One of these contains a quantity of mercury, and when it is raised above the other, the mercury seeks to pass into the lower vessel, which is connected with the injecting bottle; the air in the second vessel being compressed in proportion to the height of the first, the pressure can be easily regulated. The support is graduated, and the pressure is indicated by the number of centimeters the one vessel is higher than the other.

It is necessary to take care before injecting that there is no air in the tube between the injection-bottle and the cannula. Both this bottle and the animal are placed in warm water.

An equal pressure can be obtained by a very simple arrangement.

A vessel containing the injection mass is provided near its lower end with a tube of some length, to which a cannula is fitted. The fluid is allowed to completely fill the tube, which is then closed by a clip. The cannula being secured in the vessel the clip is removed and the contents are driven forwards by the simple weight of the fluid in the vessel, the pressure being determined by the height at which it is placed.

Of the various apparatus used in special kinds of microscopic work we may mention the **dissecting microscopes** of the different makers. In teasing out and isolating elements preparatory to an examination by a higher power, these instruments are very useful. An ordinary lens will be found better than the naked eye, and may be suspended over the object by any convenient and simple method.

The **moist chamber** of Recklinghausen is formed by fixing a glass ring on a slide. The object is placed inside this ring, and a cover-glass is put over it. A thin caoutchouc tube is fastened round the ring below, and the objective or tube of the microscope above. Some pieces of wet blotting-paper are placed inside the chamber thus formed. Schultze's improvement consists in substituting a glass cylinder for the caoutchouc, the upper part fitting the tube of the microscope, and evaporation prevented by inserting a piece of leather between the two.

A simpler method still of protecting a preparation from evaporation consists in modelling a ring on the slide with wax. Some pieces of wet filter-paper are placed within it close to the wax. The object is placed on a cover-glass which is then laid on the wax cell, the object being on the under surface.

By perforating a ring in two opposite places and fitting the apertures with tubes, a current of gas may be passed over the preparation.

There are several forms of **warm stage** in use. Only those possess much value by which the object under examination can be kept for a considerable time at the same temperature. This is best effected by placing the object over a box, perforated in the centre to admit light from the reflector. The box is by a special arrangement kept full of hot water, the temperature being registered by a thermometer.

Stricker and Sanderson's apparatus, in the form in which it is now made and sold by Mr. Hawksley, Oxford Street, London, fulfils these conditions well, and can also be used as a gas-chamber. The forms described by Mr. Schæfer ("Course of Practical Histology," p. 22), sold by Casella, Holborn Bars, and by M. Ranvier ("Traité technique d'Histologie," p. 41), sold by Vérick of Paris, are also well adapted for the same purpose.

Examination of tissues by polarised light is effected by a **polarising apparatus.** This consists of two Nicol's prisms, one, the polariser, being placed between the mirror and the object; the other, the analyser, being placed above the ocular. When the analyser is turned so that its plane of polarisation is at

right angles to that of the polariser, the field is dark; but when they are parallel the light passes through the analyser. If now, when with crossed Nicols and a dark field an object that refracts light doubly is placed under the microscope, it appears luminous. This property of refracting light doubly is possessed by several tissues, and has been much used in the study of bone and muscle.

It would appear from studies made by Ranvier that this property of double refraction possessed by tissues does not indicate more than some unknown modification of their molecular arrangement. He cites hyaline cartilage in support of the view that it depends on the condensation of the constituent molecules of the tissue, the same element refracting singly in one stage of development and doubly at another.

Preparations obtained by teasing out portions of tissue with needles may be successively washed with water, stained, again washed, and finally preserved in glycerine without moving them from their position on the slide, the fluids being removed successively by means of filter-paper. Or if a cover-glass has been put on, one fluid may be made to give way to the other by placing at the edge of the cover-glass a drop of the fluid which is to be substituted, and a piece of bibulous paper at the opposite edge. The same process may be used for sections. These are however usually treated in a different way.

Sections of hardened soft tissues, or of cartilage in its natural condition, or of softened bone are thus made. A portion of the tissue is held between the thumb and forefinger of the left hand, and is kept moist by spirit. With the razor or knife wetted with spirit an even surface is made with a rapid stroke. Thin sections are then made by drawing the knife slowly from heel to point, the instrument being supported and guided on the forefinger of the left hand. A vessel containing alcohol is prepared, and the knife is frequently moistened in it in order to keep a supply of fluid on the hollow surface of the blade. The sections, when very thin and delicate, are floated from the knife to a watch-glass or slide as the subsequent manipulations

may render convenient. When the consistence of the tissue permits they can be removed from the knife with a needle.

With practice it is possible to obtain sections by this simple method, which cannot be surpassed.

To be able to cut good sections with a free hand is of great importance in histological work, but requires considerable practice. The beginner cannot do better than continue to cut sections of intestine, liver, or skin, hardened by being placed for a day or two in $\frac{1}{2}$ per cent solution of chromic acid and subsequently for 24 to 48 hours in alcohol, until he becomes an adept.

It is often impossible to hold small portions of tissue or minute organs in the hand without injuring them. They are then embedded in a substance which is fluid at a comparatively low temperature and solid at ordinary temperatures.

For this purpose a paper **mould** or **trough** is prepared somewhat similar to the soufflé cups and ramequin cases supplied by stationers to pastry-cooks. It can hardly require much ingenuity to construct such a mould with a piece of stiff paper. How it can be done will be at once seen by taking a piece of ordinary writing-paper 4 inches by 3 inches. Make two lateral folds, an inch broad each, bringing one fold over the other, and then fold each end $\frac{3}{4}$ inch. Unfold the paper, and bring up the sides and end parts at right angles to the centre. To complete the mould it is necessary to make further diagonal foldings at the corners or to use scissors and gum. A small capsule of thin sheet-lead may be made once for all.

An **embedding mass** is made by melting pieces of white wax with olive oil in a porcelain capsule.

When the part which is in contact with the capsule begins to solidify the mass is ready for use, and should be poured into the trough.

A mass that is too hard should be remelted and olive oil added; if too soft, wax should be added. It is convenient to have a harder mass in summer than in winter.

The tissue to be cut must be embedded in such a position

that sections made at right angles to the long axis of the trough will be in the direction required. There are two ways of fixing it in the mass. The trough is placed on a piece of cork or soft wood. The object is placed on bibulous paper until nearly but not quite dry, and transfixed with a needle or pin, which is then pushed through the trough at one end into the cork. This done, the melted mass is slowly poured into the trough at the other end. The object must be so fixed that it shall be completely covered. When the wax is sufficiently hard the needle is loosened by a gentle rotatory motion and withdrawn. The exact position of the object may be indicated by a line drawn on the surface of the mass whilst it is semi-solid. This is often necessary where, in order to obtain sections in various directions, several portions are embedded in different positions in one trough.

If it is advisable to avoid injuring delicate tissues by pushing a needle through them, the following method is used: Fill the trough half full with melted mass. When this is nearly firm place the object on it, and when it has become adherent to the surface add slowly more melted mass until the embedding is complete. In this case the position of the object must be at once noted, as there is no needle-mark to fix it.

When the sections are cut the mass is first sliced off with an ordinary scalpel until the object is nearly laid bare, and the section-knife moistened with alcohol is then used. The surface of the object must not be allowed to dry, and if the operation of cutting is intermitted the mass can be inserted into a vessel containing alcohol.

Paraffin, either pure or with a small proportion of lard, is also used as an embedding mass.

Transparent soap without glycerine has been introduced by Flemming[1] as an embedding mass, when it is desirable that the object shall remain visible. The soap is dissolved in warm alcohol. Lavdovsky recommends that the sections should be made as soon as the mass has cooled.

In Ranvier's laboratory tissues are often embedded in elder-

[1] "Archiv. f. Mik. Anat.," vol. ix. 1873, p. 121.

pith. The object, when slender, is placed in a hole which is slightly larger than itself, and the pith with its contents put in water or other fluid according to circumstances. The swelling which it undergoes firmly fixes the portion of tissue. Or, when the action of alcohol is not injurious, the object is gummed on one surface and applied to the elder-pith. The whole being then placed in strong alcohol, the gum solidifies, and sections through the pith and tissue can be made.

When it is desirable to have very large or a number of consecutive sections, a **microtome** is used. Ranvier's microtome is shown in fig. 2. A circular piece of cork is inserted into the tube, which it should accurately fit, and the object is embedded in the usual way, paraffin being in this case preferable. By slight turns of the screw the mass with its object is brought to the level of the platform, and sections cut in the usual way.

Fig. 2. Ranvier's Microtome.

Mr. Stirling's microtome is larger, and is fastened to a table by a screw.

Dr. Rutherford's **freezing microtome** has a metal trough which surrounds the cylinder, and contains a freezing mixture of equal parts of snow or pounded ice and salt. The tube is filled with thick solution of gum, and the tissue held in it until it becomes fixed.

The gum is dissolved out of sections of hardened tissue by diluted alcohol, and of fresh tissue by salt solution.

Tissues which have been hardened in alcohol are not fitted for this process until they have been first immersed in water.

It is always of advantage to allow the tissue to soak in gum before freezing it.

Another method of freezing is to wrap a piece of paper round one part of the portion of tissue, and then place it in a platinum capsule, surrounded by pounded ice and salt. The knife is cooled by being laid on the same mixture. When the tissue is hard it is taken out with forceps and the paper-

covered end held in the left hand. Sections are rapidly made, and as soon as the tissue thaws it is put back again to freeze.

The simplest method yet devised seems to be that recommended by Dr. Urban Pritchard.

A solid cylinder of copper with flat ends, $1\frac{3}{4}$ inch wide and 3 inches long, is cooled in the freezing mixture. It is then wiped quite dry with a cloth and enveloped with thick flannel, one end being left exposed. A drop of gum is placed on this end, and the tissue to be cut placed on the gum. They are both speedily frozen, and sections are made with a dry knife cooled in the mixture.

Sections from frozen fresh tissues are examined in serum or salt solution. They can also be stained by gold and silver solutions.

Sections of tissues which have been hardened in chromic acid and alcohol are usually "mounted" or **preserved in glycerine**, or Canada balsam, or dammar varnish. If they are to be mounted in glycerine, they are first placed in water for five or ten minutes, and then transferred to a drop of glycerine placed on the centre of a slide. If they are to be **stained**, they are transferred from water to the staining fluid until sufficiently coloured, when they are again placed in water for a few minutes before being mounted. They may be stained by being placed either for a short period in a strong solution or for a longer period in a weak solution, the choice of the method depending on the tissue and other conditions. The process of the staining can be accurately followed by placing a section from time to time in water in a watch-glass, and examining it with a low power.

Chromic acid sections stain better if they are allowed to macerate in distilled water until their paler colour shows that they have parted with some of the acid. The fluid in which a tissue has been hardened has an important effect on the readiness with which staining is effected by different dyes.

In mounting in glycerine air-bubbles should be avoided as much as possible. If small or few in number they do not

spoil the preparation, but they produce a disagreeable impression of slovenliness. By a little care a preparation need never be disfigured by an air-bubble. The following precautions are effectual. The small bottle from which the drop of glycerine is taken should not be filled more than three parts full to begin with, and the glycerine, instead of being allowed to fall into it, should be made to flow slowly down the side. Before being used any air-bubbles which have been produced in filling the bottle should have disappeared. The glass rod should never be plunged quickly into the glycerine, but dipped into it gently, and withdrawn in the same way. It is then held obliquely over the slide, and the point brought almost or quite in contact with its surface. The drop must never fall from the rod to the slide, but be allowed gradually to flow from the one to the other. When the drop is sufficiently large the rod is slowly withdrawn. The preparation is placed in the same gentle manner, edgeways if possible, in the glycerine. If it remains on the surface of the drop it must be carefully depressed with a needle. Similar precautions should be taken with the cover-glass. It must never be allowed to fall on the glycerine. Supported by one edge on the slide and the other on a blade of the forceps, it is brought slowly down to the fluid.

The glycerine should usually be just in sufficient quantity to extend on all sides to the edge of the cover-glass. If it is in excess some of it should be removed by a small piece of filter-paper. The glycerine which is left by the paper being carefully and efficiently wiped off, the preparation can be made permanent. This is done by fixing some cement on the margin of the cover-glass. Various substances are used for this purpose. Dammar varnish soon dries and answers the purpose effectually, if the preparation is not much disturbed. We have found Brunswick black more reliable, and recommend it for the purpose. It is sufficiently fluid to flow freely from a small glass rod, and rapidly dries.

It is sometimes desirable to inclose a portion of tissue of

some thickness, so that there is a surface of glycerine exposed between the cover-glass and slide. If Brunswick black is used rapidly and freely, such preparations can be easily preserved. If it has become thick from keeping, a little turpentine should be added.

Sealing-wax softened in alcohol can also be used.

When a section is to be preserved **in Dammar varnish, or Canada balsam,** it is treated as follows. If previously hardened in alcohol it can be placed again in alcohol for a few minutes as soon as it is cut. If it is to be stained it is placed first in water, then in the staining fluid, again in water (unless it is stained in an alcoholic solution of the dye, in which case it does not require to be put in water), and then for ten minutes in ordinary, or better, absolute alcohol. This is done because it is necessary to free it from water. From the alcohol it is transferred to oil of cloves or turpentine (the excess of alcohol on the preparation being first allowed to evaporate). Oil of cloves is the more pleasant to work with of the two, and is generally used. In a few minutes the preparation becomes transparent, and is then placed on a slide in a drop of dammar. A cover-glass is put on, and the preparation is permanent.

In most instances glycerine is preferable to dammar, especially for the cellular elements. But there are occasions in which it is advisable not to bring the hardened tissues in contact with water, and for these dammar is available. Dammar is also well suited for preparations in which the nerves are stained by gold or osmic acid.

Canada balsam is prepared for use by dissolving it in chloroform.

Dammar varnish is thus prepared: One part of gum dammar is dissolved in two parts of oil of turpentine, and one part of gum mastic in two parts of chloroform. Mix the solutions, and filter.

Osmic acid preparations may be preserved in a nearly saturated solution of acetate of potash, as well as in glycerine. The glycerine used for preparations which have been stained

in carmine should contain one per cent. formic acid, or be slightly acidified by acetic acid.

Fresh tissues can be preserved for a time in glycerine, and by the addition of colouring fluids to it they can also be stained in it. This method is adopted when it is necessary to avoid the action of water. As pure glycerine shrinks fresh tissues by withdrawing water from them, it is advisable to place them first for some hours in a mixture of equal parts of glycerine and distilled water.

Sections of some fibrous tissues when placed in oil of cloves or turpentine, curl and become so brittle, that it is impossible to spread them out flat on the slide. This is avoided by placing the section on the slide direct from the alcohol, and as soon as it is nearly, but not quite dry, putting a cover-glass over it. The oil of cloves is allowed to pass in between the glasses, the weight of the thin cover being sufficient to keep the section flat.

Thick sections which have been made transparent in oil of cloves do not keep well in dammar, as they lose their transparency after a time. Exner recommends that they should be put up in a mixture of one part oil of cloves and two or three parts mastic.

BLOOD.

The drop examined should be so small that it barely suffices to fill the space between the object and cover-glass.

The nuclei of the **red corpuscles of the frog** can be stained by dyes like the nuclei of other cells. It has been pointed out by Ranvier that when frog's blood has been treated by diluted alcohol and stained by aniline, a substance on the surface of the corpuscle is especially coloured.

The hæmoglobin of the corpuscle of the frog may separate from the other parts of the blood-cell, and assume special forms within it. This can be observed by sealing blood serum containing a few corpuscles on a slide with Brunswick black, and examining the preparation daily. The changes take place after an interval of several days.

To observe the form of the red corpuscles of the frog press gently on the cover-glass with a needle. Some of them roll over under the pressure. In the centre of the discs which are seen edgeways the nucleus can be seen projecting on either side. The projection of the nucleus masks the form of the corpuscle, which is otherwise that of a bi-concave disc.

Observations on red corpuscles which are passing through the walls of capillary vessels in inflamed transparent tissues, show that they possess a small degree of elasticity. A corpuscle is sometimes torn in two by the force of the blood-current when one part of it is fixed in the wall. The separated portions assume a globular shape.

To observe **mammalian blood** prick the finger with a needle and examine a small drop. The red discs run together in the form of piles. Separate them by pressure and they run together again in a new series. It is thus seen that they are not agglutinated. Examine defibrinated blood. The piles are seen in it also, which shows that their formation is independent of the fibrine. In a short time after the preparation has been made many of the corpuscles are seen to assume a spiked or crenated form. This is caused by concentration of the serum. Make them roll over by pressure on the cover-glass, and it is seen that they are bi-concave discs. As the microscope is focused the centre of the flat disc appears dark when it comes first in view. By depressing the focus further it becomes clear. The cause of this is to be found in their shape. Place a drop of water at the edge of the cover-glass so that it can mix with the blood. The corpuscles become colourless and spherical.

The changes produced by contact with acids and alkalies, ether, bile, and solutions of urea may be observed by a similar process.

Contact with reagents may be hastened by placing a strip of filter-paper at the side opposite to that at which the drop of fluid has been placed. The abstraction of fluid by the paper is quickly replaced from the drop.

Delicate filaments of **fibrine** may be observed with a high

power in human blood. Minute particles, single or sometimes agglutinated, showing molecular movements can also be observed under favourable circumstances. They are seen in great numbers in the blood of cachectic persons, and have in such conditions been mistaken for bacteria. Their nature is not known.

The red corpuscles of mammalia (man, rabbit, guinea-pig) are stained by eosin a special rose-orange colour. In vertebrata which have nucleated blood corpuscles (hen, frog) eosin stains only the plasma of the corpuscles, the nucleus remaining colourless (Wizzozky).

In a drop of blood, in addition to the great number of red corpuscles, several **colourless corpuscles** can be detected if carefully sought for. To examine these bodies satisfactorily it is convenient to take a drop of lymph from one of the lymph-sacs which are found under the skin of the frog. Or let a drop of frog's-blood coagulate on the under surface of a cover-glass, evaporation being prevented in the following manner: Fasten a glass ring on an ordinary slide. Moisten the inner surface of the ring and place a narrow strip of wet blotting-paper inside (the simple moist chamber). Then lay the cover-glass on the ring, with the drop of blood on its under surface. As the coagulum forms colourless corpuscles are seen in the serum on its edge. Observe them closely and it can be observed that they change their form slowly. It is also seen that the corpuscles in lymph and the colourless corpuscles in blood are identical in appearance. (It is admitted that they are the same structures.) The movements referred to are the so-called amæboid movements. They can be seen for a short time in a drop of blood freshly drawn from a vein. They are more active when examined on a warm stage at the temperature of 36° to 38° C.

At a temperature over 50° C. for mammalia, and 40° C. for the frog, they die and assume a round form. A large number have a single round nucleus, but it generally falls into several pieces by the use of reagents.

The colourless corpuscles have the power of taking minute insoluble particles into their interior. Cohnheim found that when insoluble aniline blue in a state of fine division is injected into the lymph-sac of the frog, it is seen after a time in the corpuscles present in the interstices of the tissues of distant organs.

The addition of solution of iodine demonstrates the existence of glycogen in some colourless corpuscles by staining it a mahogany brown.

The nuclei stain in logwood and may also be readily seen by the addition of water. Examine dead cells and observe that they have become more granular. (At the same time a drop of pus may be examined, when it will be found to contain a large number of round granular cells which are indistinguishable from dead lymph cells).

To see crystals of **hæmoglobin** place a drop of blood (that of the guinea-pig is said to answer best) on a slide, and let it evaporate until it is nearly dry. Then add a drop of water to the blood, place a cover-glass over it, and let it dry slowly. As it becomes dry the crystals appear.

To see **hæmin** crystals let a drop of blood dry on a slide. Add a very little finely-powdered common salt, and then moisten the whole with one or two drops of glacial acetic acid. Cover immediately with a cover-glass in order to keep the acetic acid together. Place a hair between the glasses to gain space for the acid. The slide is heated over a spirit-lamp so quickly that the acetic acid begins to form bubbles before it has had time to evaporate. (The breaking of the glass is to be guarded against.) The preparation is then ready for examination. Most crystals are found at the edge of the cover-glass or near the hair.

M. Malassez has invented an apparatus for **counting the corpuscles** in a given quantity of blood.[1] It consists of two instruments, one for diluting the blood to a known degree, and

[1] See further "Archives de Physiologie," 1874, p. 32, or "Traité technique d'Histologie" (Ranvier) p. 204.

another for counting the number of corpuscles in a known quantity of the diluted blood. The first is a capillary tube ending in a bulb. Numeral figures are engraved at either extremity of the bulb. The blood enters the tube by capillary attraction or is drawn into it by aspiration to the figure 1. An artificial serum is then immediately drawn after it to the figure 101; and by a combined rotatory and up-and-down motion of the tube, and the consequent movements of a small glass ball in the bulb, the blood is thoroughly mixed and diluted 100 times.

The other instrument is for counting the corpuscles. A capillary tube is fixed on an ordinary glass slide. On this slide have been scratched figures indicating the fraction of a millimeter cube, which is represented by a given length of the tube in micro-millimeters, the lengths chosen being usually 600, 500, 450, 400 micro-millimeters, the fraction calculated for three or more of these lengths being usually marked.

In order to count the corpuscles in this tube a certain arrangement of the microscope is necessary. A micrometer eye-piece, marked in squares, is used; and an objective is selected of such a power that the micrometer eye-piece covers on a stage micrometer one of the lengths in micro-millimeters marked on the slide with the capillary tube. The tube of the microscope is drawn out until the length of the micrometer square coincides with the lines on the stage micrometer, and a mark is made on the tube to indicate the exact point to which it has been drawn and the number of micro-millimeters measured.

This done the corpuscles can be counted. A drop is blown out of the mixing instrument by a caoutchouc tube attached to its other end, and, being necessarily almost pure serum, is rejected. Part of a subsequent drop is allowed to enter the counting tube by capillary attraction, the fluid at its end being gently moved until the tube is filled in order that the blood may remain thoroughly mixed. This done, the blood external to the capillary tube is carefully removed by blotting-paper,

and the tube of the microscope being drawn to the point previously marked on it, the corpuscles within the limits of the square in the micrometer are counted, the process being much facilitated by the smaller squares into which the micrometer is divided. The number being ascertained, it is multiplied first by the number on the slide opposite to the number of mikromillimeters in the length of the capillary tube which have been counted. This gives the number of corpuscles in a cubic millimeter of the diluted blood. The product is again multiplied by 100 to rectify the dilution, and the product is the number of corpuscles in the blood in its natural condition.

It will be seen that it is necessary to use always the same microscope and the same objective. A magnifying power of about 100 diameters or slightly more is convenient.

The artificial serum recommended by M. Malassez is composed thus:

Solution of gum arabic giving, by an urinometer, sp. gr. 1·020—1 volume.

Solution of equal parts of sulphate of soda and chloride of sodium, also having sp. gr. 1·020—3 volumes.

With this serum it is necessary to operate quickly, so that the blood may be thoroughly mixed with it before the corpuscles have run into *rouleaux*.

Mr. Schäfer has suggested ten per cent. solution of sulphate of soda as an artificial serum, and this fluid has the advantage that it completely prevents the corpuscles adhering to each other. It has the disadvantage that they continue to move slightly for some time, and that they rapidly accumulate in the centre of the tube, both of those disadvantages being obviated by the gum in the former mixture. For those who have not acquired dexterity in operating, we recommend the sulphate of soda solution.

There are sources of error in the method that it is impossible to completely avoid, but in the hands of the same operator the same instrument gives fairly uniform results, and valuable comparative data can be obtained by it.

The average number of corpuscles in human healthy blood is five millions per cubic millimeter. In cachectic conditions it descends as low as two millions.[1]

Squamous epithelium can be conveniently observed *in situ* by examining the anterior surface of the cornea of a frog fresh or in ½ per cent. solution of common salt. In order to examine thoroughly the individual cells, place the cornea in solution of bichromate of potash for 24 hours, and tease out the epithelium with needles. They may be isolated also after maceration in iodised serum, or 10 per cent. salt solution, and various other reagents.

Maceration for 24 hours in a mixture of 1 part alcohol in 2 parts water has been lately recommended by Ranvier, and we can testify to its great value for the isolation of the various forms of epithelial cells.

Columnar epithelium can be examined in the mucous membrane of the small intestine, and isolated by the same methods.

The free surface of columnar epithelium cells is formed by a thickened edge or border, definitely marked off from the rest of the cell. In sections or in isolated patches of columnar epithelium, smaller, more rounded, and more or less granular cells are observed between the individual cells at their base and in contact with the basement membrane. The forms of these cells support the hypothesis that they are destined to replace the epithelium which is cast off, and it is worthy of note that the new cells are never found within the old ones, nor partially attached to them. The fully developed cells are never found dividing into two cells. The younger cells then are not formed from the previously-existing developed cells.

Ciliated epithelium can be detached from the pharynx of the frog, and examined in aqueous humour. The ciliary motion is then seen.

For a more special study of ciliary motion the gill of the common salt-water mussel will be found convenient, a small

[1] The instruments and *verre quadrillé* for the micrometer eyepiece are supplied by M. Vérick, 2, Rue de la Parcheminerie, Paris.

portion of one of the bars being dissected out in a drop of the sea-water which is contained in the shell. The effect of slight increase of temperature in reviving the motion can be observed on the hot stage, evaporation being prevented by placing oil round the edge of the cover-glass. The stimulant action of very weak solution of potash can be observed by adding it in minute quantities at the edge of the preparation. The effect of carbonic acid gas alternated with oxygen can be observed in a simple gas chamber, and that of chloroform vapour by putting a drop of chloroform in a simple cell adapted to a slide by a glass ring or wax, and inverting over it a cover-glass, to which ciliated epithelium in an active condition is adherent.

The discharge from the nostrils during the first 24 hours of a nasal catarrh contains ciliated epithelium in abundance, and if a drop is placed under the microscope the movement of the cilia is seen. The cells are found in various stages of development, some of them being spherical, finely-granular bodies, with only a few cilia on one point. These are probably cells in an early stage of formation, and it is important to note that they are not found inside the larger, fully-developed cells, nor are the latter found dividing into the former. Their appearance in this discharge does not therefore justify the assumption that the younger ciliated cells are produced by the older ones.

As varieties of epithelium that of the bladder and gall-bladder may be isolated and studied, after 24 hours' maceration in bichromate of potash or diluted alcohol.

The isolation of epithelial cells is effected by needles. A drop of the macerating fluid is placed on a slide, and a small fragment containing the epithelium placed in it is teased out into smaller and smaller portions and examined. The operation is much facilitated by being done under a dissecting lens.

Compare with these forms of epithelium the delicate single layer of cells on the surface of Descemet's membrane. These should be examined fresh in aqueous humour, the inner surface of the cornea being turned upwards. By endeavouring to isolate them in the reagents which are so useful in demonstrating the cells of the epithelium on the anterior surface of

the cornea, it will be probably seen that they disappear and become destroyed in the process. Although arranged like ordinary epithelium, there is thus a difference in the nature of the cells, undoubtedly depending on the fact that they are developed from the mesoblast of the embryo.

The term *endothelium* has been lately adopted by the majority of writers to designate such single layers of cells, and the word has taken root in histological nomenclature. We will therefore use the term in this work. The word *epithelioid*—used by some writers to express the same thing—is more accurate from a philological point of view, but it has not passed into general use, and has the disadvantage that there is no noun connected with it.

Endothelial cells are usually flattened, delicate, frequently somewhat polygonal bodies, with a round or oval nucleus, and form single layers. The cells being usually somewhat rounded minute angular spaces are left between them, and as these spaces are generally filled by an albuminous substance, they are indicated in silver preparations by a black deposit of albuminate of silver. They are then described as the so-called "stomata." These are always seen in silver preparations of serous membranes, and are especially marked if the albuminous substance has not been previously removed in part by irrigation with water.

The layers of endothelium which exist in the substance of connective tissues are bound together by fibres which pass from one layer to another by piercing the intercellular spaces.

The study of connective tissue may be conveniently begun by examining **tendon.** By holding the root of the tail of a mouse or young rat firmly between the thumb and forefinger of the left hand and pulling the tip with the right, the dislocated end brings away with it a number of fine tendons. The skin of the mouse's tail gives way easily where it is fixed by the thumb-nail of the right hand. In the rat's tail it is convenient to make a circular incision through the integument with a scalpel.

These tendons are not allowed to shrink, but are brought immediately over a slide before being detached from the stump of the tail. They are fixed to the glass by two drops of melted sealing-wax, the space between the drops being slightly greater than the length of the cover-glass. The tendons are then divided outside the fixed points, one or two drops of picrocarminate are placed over the central part of the tendons and a cover-glass is put on. After half an hour a current of distilled water is drawn between the glasses by placing a strip of filter paper at one side until the picrocarminate is removed. Glycerine is then drawn between them in the same way until it has replaced the distilled water. The glycerine should be slightly acidified by acetic acid or formic acid (one part formic acid in 100 parts glycerine). This is the method originally recommended by Ranvier. The tendon if now examined is seen to be composed of straight parallel bundles, and arranged in rows between the bundles nucleated cells can be observed. The large rounded or slightly oval nuclei are very distinct, as are also those borders of the cells which are transverse to the long axis of the tendon. The nuclei are not in the centre of the cells, but are so arranged that contiguous pairs of cells have the nuclei near each other. The lateral borders of the cells are not usually seen in these preparations.

These cells are comparatively easily demonstrated, as compared with other cells in connective tissue. They can be observed by various other methods, amongst which the following may be recommended: Prepare logwood-glycerine by dropping logwood solution into glycerine drop by drop until the latter has acquired a very decided blue colour. Place a streak of this logwood-glycerine lengthwise on a slide, and having drawn the tendons from a mouse's tail to a convenient extent, without extracting them entirely, place them carefully and still extended in the logwood-glycerine. After a few minutes, when the tendons have imbibed sufficient glycerine to prevent their shrinking, separate them at both ends and place them in a watch-glass partially filled with similar logwood-glycerine. Let

them remain in it for 24 hours or longer. Portions cut off with sharp scissors are then placed for a few seconds in pure glycerine, and finally examined in glycerine. The cells and nuclei are visible. The transverse borders of the cells are again distinct, but the lateral borders are still badly defined. It will now be seen, if the preparation is a successful one, that the rows of cells are more numerous than they appeared to be in preparations in which the cells are stained in the usual way by carmine. The rows which correspond to the wider spaces between the bundles are most conspicuous, but if there has been much staining, faintly coloured rows can be detected between these. If the cells are further examined, they are seen to be marked longitudinally with blue striæ, and when such a stripe is in a line with the nucleus it passes through it.

The best marked of these striæ correspond to ridges in the cells. Ranvier, who has pointed this out, recommends for its demonstration that the tendons be placed in one per cent. solution of osmic acid for 24 hours, then in picrocarminate for 24 hours, and examined in glycerine containing one per cent. formic acid. An attempt should be made to isolate the cells. Histologists differ as to whether these cells completely invest the bundles, or whether they are inserted amongst them in isolated rows. They are agreed that they are applied to the surface of the bundles. In connection with this question, in addition to the logwood-glycerine, we recommend the following method: The partly extracted fresh tendons are laid on the slide in $\frac{1}{2}$ per cent. solution of chloride of gold for a minute. They are then separated and cut into small pieces on the surface of the glass by pressing the edge of a scalpel on them. These portions are placed in a fresh drop of gold solution, and Brunswick black run round the edge of the cover-glass seals the fluid. After 2 or 3 to 24 hours they should be carefully examined. Although there is no staining produced in the ordinary sense the cells are fixed and are visible. Their outlines are better defined than by previous methods, and the number visible is sometimes surprising.

Successful preparations obtained by these methods show, as we believe, that the cells are not in isolated rows, but that the bundles are completely invested by them. The appearance of isolated rows is due to the fact that by the usual methods only those cells are stained or hardened which invest that surface of a bundle which is free towards the spaces which are naturally produced by the juxtaposition of parallel cylindrical bodies.

It is almost impossible to isolate these cells by the ordinary methods of teasing out by needles. The structural elements which are thus usually obtained, and which have been confounded with the cells, are produced thus. Between the cells and the bundles there is an apparently structureless sheath, and between this sheath and the bundles, or in the sheath itself perhaps, there are elastic fibres. Further, the sheath is continuous with prolongations or septa which pass inwards between definite subdivisions of the bundle. The cell is intimately applied to the surface of the sheath, and unless loosened from it by chemical agents, cannot be separated from it. Morphologically, however, it forms no part of it. Again, the elastic fibres are so intimately adherent to the sheath that they are inseparable from it by merely mechanical means.

Further, there is in or on the surface of the sheath and the septa that pass from it a substance that stains by carmine and logwood, and by which chloride of gold is freely reduced. A small quantity of the same substance surrounds the elastic fibres and follows them in their course.

These conditions produce appearances that are very misleading. Attempts at isolation of the cells, if these have not been previously loosened from their connection, are generally answered by the separation of a part of the cellular membrane, on which the nucleus of the cell and more or less of the cell substance are visible, and with it probably some elastic fibres enveloped and concealed in shreds of membrane. Hence the idea of a cell with irregular contours and fine prolongations. If some of the septa are removed with the membrane the appearance is still more complicated.

There is a substance within the cell which stains by dyes and reduces gold in a manner similar to that which surrounds or bathes the elastic fibres, and, as the latter are immediately under the cells, we have an appearance of cells with processes again simulated, and this all the more as the fibres themselves are very rarely visible.

The appearances seen in gold and logwood preparations, which have been interpreted as cell processes, are nothing more than stained extra-cellular amorphous matter which surrounds the elastic fibres.

There are thus two kinds of striæ produced in tendon by dyes and reagents. One is nothing more than ridges in the cells (*crêtes d'empreinte*, Ranvier), the other are long narrow lines of stained matter which follow the course of the elastic fibres. The latter are seen in gold preparations of tendon, in which reduction has been favoured by an unusually strong solution of acetic acid.

The cells successfully isolated by Boll and Ranvier have no processes, but are somewhat oblong bodies with regular edges. Our own observations are in accordance with the results obtained by these investigators.

Place a small fragment of tendon in 1 per cent. solution of osmic acid, or in a saturated solution of picric acid for 24 hours, and then tease out in glycerine. The tendon bundle is seen to be composed of a great number of extremely fine cylindrical threads. This may be demonstrated in another way. Cut out the part of the tendo Achillis of the frog between the muscle and the sesamoid bone. Split it longitudinally with scissors into several pieces, and seal them on a slide in blood serum with Brunswick black. Examine in 2 to 3 days, and the delicate cylindrical threads of the tendon are distinct.

Place a portion of a mouse's tail in saturated solution of picric acid for 2 to 3 days. Embed and make transverse sections. Stain them in picrocarminate. The tendon sections are cut up by stellate fissures and finer lines which connect them. Nuclei are visible in the spaces. These are the nuclei of the cells seen by the previous methods, and the part of tendinous substance enclosed by the spaces corresponds to a bundle. Around the surface of the bundle there is decided carmine staining, and similar stained points are seen within the bundle.

The former appearance is supposed by some histologists to correspond to a sheath, and the points to fibres that take their departure from it and penetrate the bundle (Ranvier).

Place a part of the tendo Achillis of the frog (between the sesamoid bone and the muscle for example) in $\frac{1}{2}$ per cent. gold solution for 20 to 30 minutes. Then expose it to light in distilled water, acidulated slightly by acetic acid (1 to 2 parts in 100 water) for several days or until it is dark in

colour. Cut it into two portions. Embed them. Cut one into transverse sections and the other into longitudinal sections. In the longitudinal sections rows of the large flat cells are seen characteristically stained. In the transverse sections the stellate figures are well marked, being indicated by a dark finely-granular substance. The contour of the bundle is not always definite, and within the bundle a number of dark points are visible. These dark points indicate that the bundle is not a solid homogeneous mass, although it does not necessarily follow that the points are cell-processes as has been supposed.

The matter seen between the bundles in transverse sections which stains in carmine and is reduced by chloride of gold, is not the body of the Ranvier tendon cells. It is probably partly due to a substance contained in the structureless subcellular sheath; but we believe that it is, if not entirely, at least to a great extent, produced by amorphous matter in the lymph fluid which occupies the interstices of the bundles.

Elastic fibres are seen in tendon after prolonged boiling.

So much concerns facts regarding tendon which are not particularly difficult of demonstration, and which are mentioned more or less by most of the authors who have lately written on the subject. There are other questions which invite investigation, but which are hardly suitable to a beginner. One of these is the nature of the layers of areolar connective tissue between groups of bundles. Others concern the further analysis of the bundles and hitherto unnoticed cellular structures described by ourselves.

When transverse sections of the frozen fresh tendo Achillis of the calf are examined in $\frac{1}{2}$ per cent. salt solution, the surface of the bundle is seen to be mapped out into small polygonal fields—similar to Cohnheim's fields in sections of frozen muscle (Herzog).

In longitudinal strips of tendon sealed several days in serum, or treated by logwood glycerine, and by various other methods, lines are seen which indicate that the bundle is composed of smaller definite cylindrical structures. In mammalian fœtal tendon preserved for a lengthened period in $\frac{1}{8}$th per cent. solution of chromic acid (the attachments at both ends being preserved), we found the tendon composed of fine cylindrical structures which in transverse section were arranged in groups, which indicated the formation of the ordinary bundle.

In logwood-glycerine preparations some of the striæ on the ordinary large tendon cells correspond in relative position to the lines on the bundles alluded to, and which we believe indicate the borders of component parts of the bundle. They are seen on the cells in the accompanying woodcut.

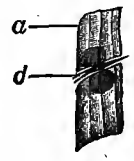

Fig. 3. Cells in tendon stained by logwood-glycerine: *a*, the endothelial cell of the bundle; *d*, cell lying transversely between the flat cells.

We have on several occasions observed small narrow elongated cells in tendon, about the length, and a fourth or fifth part the width, of the ordinary tendon cell, namely, in the mammalian fœtal tendon hardened in weak chromic acid, on fragments of tendon macerated in blood serum, and in gold preparations of tendon in an early stage of inflammation. In the chromic acid preparations they distinctly covered the narrow cylindrical subdivisions of the bundle, which we have elsewhere termed primary bundles. They were observed only in a few instances during an examination of tendon carried on for a considerable period of time, and we know of no method by which their demonstration can be readily guaranteed. The same remark applies to the question of the existence of branched cells, as distinguished from the cells which are applied to the bundles or *Häutchenzellen* of Key and Retzius. We have described cells which are characterised by a small oval nucleus, a very scant amount of cell-substance, and very fine cylindrical thread-like processes which pass between the bundles. The nucleus and cell-substance of this branched cell may be sometimes observed in logwood-glycerine preparations (more rarely by other modes of preparation), lying on the bundle transversely between two of the ordinary or Ranvier cells.

Fibres, which are evidently elastic fibres, can be shown in tendon by injecting ¼ per cent. solution of chloride of gold through the bloodvessels. The tendo Achillis of a young rabbit after injection from the femoral artery is suitable for this purpose. Pressure should be kept up for some minutes, so as to secure penetration by the solution, and longitudinal sections made either immediately or after hardening for 24 hours in alcohol.

What is called **areolar connective tissue** may be examined by cutting out a small portion with scissors, from the subcutaneous tissue—from the mouse's back, for example. Examined in water, it is seen to be composed of waving or twisted fasciculi. By adding acetic acid elastic fibres are seen.

If the tissue is hardened in alcohol and stained in logwood, nuclei are seen, indicating the existence of cells. Various opinions are held as to the nature of these cells, and they are very difficult to demonstrate. The following method we have found successful: Kill a mouse, and the same instant inject ¼ per cent. solution of nitrate of silver under the skin of the back. Or, chloroform a mouse, inject, and after a couple of minutes kill it (whilst still anæsthetised). Carefully but quickly dissect the skin from the subcutaneous tissue. Wherever the silver solution has penetrated, the latter is white and œdematous. Cut off small portions of it with sharp scissors (taking care not to press or drag it), and place them on a slide in a large drop of glycerine. Put on a cover-glass, and expose the preparation to bright sunlight. As soon as the tissue begins to darken, examine it. When the method is successful rounded or oval nucleated cells are seen, the cell substance being darker than the other tissues. At certain parts where the staining has been very complete, numbers of the cells may be seen to form a layer, small openings being left in the angular spaces between the cells. These cells have no processes, the border of the cell being cleanly cut all round. After several portions have been removed by scissors, preserve the rest for further examination by immersion in glycerine.

The whole operation must be carefully and quickly done, and repeated if necessary until a successful preparation is obtained.

The demonstration of these cells is of great importance, as it proves the existence of layers of endothelium in areolar tissue, and gives a key to the further investigation of all the forms of connective tissue. The success of the method depends on the fact, that when endothelial cells are brought in contact with solution of nitrate of silver before *post-mortem* changes have taken place—such as, for example, would be produced if they were previously treated by water—the cell substance combines with the silver, and an albuminate is

formed within the cell. (See "Proc. Roy. Soc.," vol. xxii., plate ix., fig. 8.)

The same cells can be seen by treating portions of the fresh tissue by the saturated potash solution, but success is very rarely obtained by this method. When it does succeed, however, narrow elongated cells are sometimes also found isolated, showing that the endothelial layers of rounded cells are not the only flat cells in the subcutaneous areolar tissue.

The method of interstitial injections in the study of connective tissue was introduced by Ranvier, who also recommends the injection of a small quantity of warm melted gelatine. The gelatine is allowed to swell in water for an hour. It is then washed clean and melted over a water-bath. The œdematous bulla soon sets, and, when hard, sections are cut. These can be stained and examined, the interstices of the tissue being distended by the gelatine. But the results by this method do not equal those we have just described as obtainable by the simple injection of silver solution.

The tissue may be also examined by cutting out fresh portions and examining them in aqueous humour, salt solution and other fluids. It is almost impossible, however, to avoid injuring it irretrievably in the process. The nuclei of the cells often persist and can be stained, but the contours of the cells are generally indistinguishable. As regards the form and arrangement of the cells the method is untrustworthy.

The **serous membranes** may be examined in sections, which include the peritoneal covering of the diaphragm, but are most conveniently studied in transparent tissues, such as the mesentery or the omentum. To examine these membranes successfully, it is necessary that they should be fresh, not allowed to become dry by exposure, and kept extended. Ranvier's *half-drying process* consists in placing a portion of the membrane on a slide, and allowing it to partially dry by evaporation, so that when extended by the fingers it adheres to the surface; a drop of staining fluid is then placed on the central portion, and a cover-glass put over it. The membrane, which should extend

very slightly beyond the edges of the glass, is again stretched by the points of the fingers, and retained in its extended position by fixing the corners of the cover-glass with paraffine. When the staining has taken effect the colouring matter is removed by a piece of filter-paper placed at the edge of the glasses, and glycerine allowed to take its place. The preparation is then ready for examination or permanent inclosure by sealing. In this process the drying must never be complete.

We have found the following method good, and recommend it in preference to any other:

Take two pieces of mica about $1\frac{1}{4}$ to 2 inches long and $\frac{3}{4}$ inch broad, one thicker and the other thinner. The necessary degree of thinness will be understood when the details of the manipulation are considered, and after a very little practice. Lay one piece over the other, and punch a circular hole through both at one end. Take, as an example, the free omentum which is attached to the greater curvature of the pyloric end of the stomach of a new-born kitten, or of a rabbit three weeks old, raise it gently with the forceps, and place the thicker piece of mica under it so that the membrane is spread over the circular hole, taking care that when once the membrane has touched the mica it is not again moved. Place the thinner piece of mica over the other so that the two holes shall exactly fit each other. An assistant then fixes the pieces of mica in their position over each other by grasping the edges with two forceps, and the omentum is detached by cutting it with scissors round the mica. The two pieces, still in contact, can then be held in any convenient position by an assistant, and they are fixed by a strong waxed thread tied round them before and behind the hole. It is advisable to prepare small notches beforehand, by which the threads can be well secured. The mica behind the holes being no longer required as a handle, is cut off by scissors, and the two-layered square of mica, with its enclosed membrane, can be easily manipulated by grasping its edges with forceps.

This manœuvre is intended to fulfil the following conditions, which are of the greatest importance in studying so delicate a tissue as the omentum. The membrane is prepared for examination in serum, or after the action of reagents, without having been unduly stretched or allowed to retract, without having become even partially dry, and without having been subjected to contact with any foreign body. The quicker the operation is performed the better. If some time is lost while it is being done, it is well to moisten the preparation occasionally with a drop of peritoneal serum.

To examine the membrane fresh a large drop of serum is placed on a slide, and the square so placed over it that the membrane shall be in contact with the fluid. Another drop is placed on the upper surface of the membrane, and a very thin cover-glass used. It is convenient to arrange so that the cover-glass shall have the same area as the square. Brunswick black run round the edges prevents evaporation, and allows the observation to be continued for hours or days. As fresh tissues sealed in serum undergo gradual changes, by which the contour of transparent elements becomes visible before they finally disintegrate, this is a matter of some importance.

Prepare a number of similar squares for treatment by the following methods:

Put them for a few hours into Müller's fluid. Then pass a camel's hair brush gently over both surfaces, the square being suspended in the fluid the while. Remove them for a few minutes to distilled water. Then stain some in carmine, some in picrocarminate, some in eosin, and some in logwood solution. After they have been again placed for a minute in distilled water, examine in glycerine, the manipulation being the same as for examination in serum. Place other squares in $\frac{1}{2}$ per cent. of chloride of gold solution 15 to 30 minutes. Then expose to the light in 1 per cent. acetic acid until the colour becomes dark. Treat others by osmic acid solution and subsequent staining by picrocarminate or logwood, or by both.

To see the outlines of the endothelium of the surface place

the square in $\frac{1}{4}$ to $\frac{1}{2}$ per cent. silver solution for a minute, then expose it on a slide in glycerine to sunlight, and examine as soon as the membrane becomes dark.

The washing in distilled water before and after the treatment with the silver solution, which is usually recommended, has the advantage of removing the albuminous matter which is adherent to the membrane, and so preventing a deposit of particles of albuminate of silver. It has, however, the great disadvantage of frequently destroying the cells. Particles of albuminate of silver, even when they are present, do not impair the usefulness of the preparations. They are mostly found in the angles between the cells, and indicate an accumulation in these points in the living tissue.

In the omentum are specially to be observed the nature and arrangement of the bundles of fibrillary tissue, the bloodvessels and lymphatic vessels, and the nuclei of the cellular elements, more abundantly visible in some parts than in others.

A method for the isolation or demonstration of the form, and arrangement of the cellular elements of the omentum is yet a desideratum. The number and distribution of large flattened, rounded, or slightly oval nuclei, justify the inference that we have here, as in the subcutaneous tissue, a layer of endothelial cells.

This inference is supported by the fact that Waldeyer has isolated rounded nucleated cells from the omentum of the mouse. The delicate nature of such cells and their firm adhesion to the tissues on which they lie constitute the difficulty of either demonstrating or isolating them.

In the omentum of young animals stained by logwood the nuclei and part of the processes of spindle cells can sometimes be observed.

In the stained omentum of young rabbits certain patches are conspicuous from the number of nuclear elements visible. These patches are also distinguishable in unstained serum preparations by a faint opacity (hence termed by Ranvier milky patches). In the first weeks of life an appearance is seen in these patches which precedes and marks out the subsequent development of bloodvessels. This consists chiefly in a very slightly granular branched figure in which nuclei exist. Ranvier believes that this figure is a cell specially destined to form a bloodvessel, and it is hence termed by him a vasoformative cell. We believe that it is produced by a distension of pre-existing spaces between the groups of bundles, and the accumulation in them of an exudation from the nearest bloodvessels,

which possesses different refractive properties from that of the surrounding tissues and which is also capable of staining. The nuclei we believe to belong to the ordinary cells of the part.

The faintly granular substance is spoken of by some authorities as vasoformative protoplasm. It is termed protoplasm simply because it is faintly granular and has special refractive properties, and vasoformative because it precedes the development of a bloodvessel. To term everything in the tissues which is finely granular and has peculiar refractive properties, protoplasm, is certain to lead to fallacious conclusions. That the appearance in question precedes the development of a bloodvessel does not show that it is cellular, nor taken alone does it show what relation it has to the vessel. It must be borne in mind that granular matter capable of staining is produced by processes of coagulation in homogeneous fluids, and that its appearance in a stellate or spindle form may be caused by the form of the cavities or spaces in which the coagulation takes place. If the walls of these spaces contain nuclei the appearance of a cell is simulated. Such we believe to be the explanation of vasoformative cells. The so-called stellate and spindle cells, which have been supposed to form bloodvessels by becoming hollow and communicating with similar cells in the vascular wall, which again are in communication with the lumen of a vessel, are to be explained in the same way. They are not cells.

We are justified in declining to accept the doctrine of vasoformative cells—of whatever presumed form—until they have been isolated.

The abundant reticulum of elastic fibres is seen to advantage by adding diluted acetic acid to the fresh membrane, and sometimes after treatment by $\frac{1}{10}$ per cent. solution of osmic acid.

Simpler, but not so accurate, methods of examining the omentum are to open the abdomen in Müller's fluid and let the membrane be suspended in it for some hours, after which pieces of it may be separated, floated on a slide, and examined by the usual methods. A similar treatment may be practised with silver solution in order to stain the substance between the endothelial cells. The membrane, after being floated to the glass, can be spread out by the half-drying process and exposed to light in glycerine, either with or without a preliminary rinsing with distilled water. The drop of glycerine must be

small and a cover-glass put on so that the membrane may be kept extended.

Fibrillary tissue may also be studied (as has been well shown by Axel Key and Gustaf Retzius) by treating the subarachnoid trabeculæ by osmic acid and subsequent aniline staining, by silver, Müller's fluid, etc., the principle being never lost sight of that until the reagents are brought into contact with the fresh tissues these must be kept as much as possible undisturbed in their natural position and relations. The demonstration of the investing cells of the bundles would appear to succeed in the subarachnoid trabeculæ more easily than in many other parts.

We succeeded once, after a considerable number of trials, in isolating from the mesentery of the frog by saturated potash solution a number of narrow elongated cells, forming a continuous layer, which have not been seen by any other method.

There is good reason to believe that there exists in the organism on an extensive scale, an extremely delicate form of fibrillary tissue, which has hitherto mostly escaped observation on account of its being destroyed by the reagents generally employed in histological research. Key and Retzius describe it as surrounding some of the bundles of the ordinary more resistant fibrillary tissue, and we have met with it in sealed serum preparations of portions of the neurilemma of the sciatic nerve of the frog, and in glycerine preparations of the enveloping sheath of the tendo Achillis of the same animal. It probably forms a large part of the connective tissue of the parenchyma of glandular organs.

We have described the fibrillary element of areolar tissue as being inclosed between layers of delicate endothelial cells. The existence of these layers of cells has been shown in the fine areolar tissue between the groups of tendon bundles by Löwe in silver, and by ourselves in gold preparations, in the testicle by Mihalkowics, and in the perimysium of the muscular fibres, and bundles of fibres of the frog by ourselves. These investigations bear out the interpretation assigned by Key and Retzius and Schwalbe to the results they have obtained in their studies of the arachnoid, pia mater, nerve sheaths, the choroid, etc., and justify the conclusion that the elementary form of fibrillary tissue consists of a membrane covered on both sides by an endothelial layer of cells, fine bundles, often of an extremely delicate

and perishable nature, being situated between the layers. The methods most likely to demonstrate the cells consist in a careful application of the silver method, chloride of gold, osmic acid, and preservation in serum. But by all these methods success is exceptional. Much depends on dexterity of manipulation. When the metallic solutions are used, a method must be chosen suitable to the particular part selected for investigation, by which the fluid can be brought into contact with the cells whilst they are still unchanged. In serum preparations they must be brought fresh on the slide, unshrunk and uninjured in any way, carefully covered, and immediately sealed by Brunswick black. They should be frequently examined during the following 48 hours.

The next stage in the development of fibrillary tissue is distinguished by a greater power of resistance to *post-mortem* change and chemical agents. The fibrillated bundles are intact after the cells have disappeared, and can be resolved into a number of exceedingly fine fibrillæ by maceration in salt solutions, baryta water, and sometimes in simple serum. This type may be conveniently studied in one of its stronger developments, such as is found in the skin and subcutaneous tissue.

Our observations on the cornea, tendon, mesentery, and our joint observations with Mr. Ewart on the retina, warrant the belief that in addition to the typical layers of cells which inclose the bundles of connective tissue, the finer bundles are themselves invested by narrow elongated cells.

Elastic fibres can be seen by adding dilute acetic acid to the subcutaneous tissue or to a serous membrane. The larger forms may be conveniently studied in the *ligamentum nuchæ*. The knowledge obtained regarding their structure and development is still very defective.

A valuable memoir on the subject has been lately published by Schwalbe.[1] This observer, in studying the fibres of the ligamentum nuchæ, subjected them to the action of 35 per cent. potash solution. By this treatment, in 24 hours they have lost their elasticity. On the second day a special form of transverse marking is observed, and soon afterwards, if they are teased out, they fall into long cylindrical fragments. Changes take place in the inner substance of the fibre between 5 and 14 days. Examined in the fluid, clear spots and

[1] "Zeitschrift f. Anat. u. Entwickelungs geschichte," vol. ii.

striæ are seen in the axis of the fibre, vacuolation beginning in the axis and spreading to the periphery. This vacuolation and destruction is hastened if water is added after the second day.

Schwalbe infers from these experiments that the substance hitherto designated as pure elastin, consists of at least two substances, a peripheral substance of the nature of a sheath and its contents. This sheath is isolable after the action of the potash, and the preparation can be preserved by complete neutralisation with alcohol, and can then be also coloured with carmine. It shrinks in the alcohol.

The sheaths are fibrillated, an appearance produced by longitudinal thickenings.

The transverse elements become conspicuous after long maceration (3 to 4 weeks) in $\frac{1}{20}$ to $\frac{1}{30}$ per cent. chromic acid. The contents break up into short cylindrical pieces. A canal forms in the centre of the fibre. If the fibres remain in the solution for several months, without the concentration being kept up by renewal of the fluid, they gradually break down until nothing but the sheath is left. Sometimes, and especially if the solution is somewhat stronger ($\frac{1}{20}$ per cent.), the sheath also breaks up into longer pieces.

The same observer identifies the transverse discs with the *grains refringents* described by Ranvier in elastic fibres. He also found them by letting the ligamentum nuchæ rot in water. He believes that the transverse discs of the larger, and the *grains* of the smaller, elastic fibres are not the primitive elements which are in his opinion constituted by the molecules of elastin.

If portions of the ligamentum nuchæ of a young animal are hardened in gold solution, and then deeply stained by logwood, the existence of numerous nuclei becomes evident. These nuclei are closely adherent to the fibres, and generally a small amount of deeply-stained amorphous matter surrounds the nucleus.

When we first demonstrated these nuclei and their close relation to the fibres in 1874, we drew the inference that they were the remnants of cells from which the fibres are developed. Schwalbe has, however, by interstitial injections of iodised serum and weak solutions of bichromate of potash isolated flat endothelial cells, of which these are the nuclei, the cell closely embracing the fibre. The fibre is therefore manifestly not a metamorphosed process of this cell. It does not necessarily follow however from this that they are not de-

veloped from cells and their processes, and although the tendency of modern histology is against this older view, rightly requiring more proof of its accuracy than has yet been given, it is safe to assert that its weakness lies in the want of sufficient knowledge of the different stages of development, and not in any satisfactory observations which support the other opinions that have been advanced.

Elastic fibres stain yellow by picric acid. When connective tissue is stained by picrocarminate, the carmine stains the bundles of fibrillary substance, and the picric acid the elastic fibres.

Ranvier states that if an œdematous bulla has been formed by interstitial injection of one per cent. solution of osmic acid into the subcutaneous tissue, the distended portion excised and placed 24 hours in the same solution, then washed in water, and fragments teased out and examined in glycerine, the elastic fibres are seen when magnified 1000 diameters to be formed by refractive lenticular or spherical granules, surrounded by a substance which is much less refractive. Magnified 400 diameters they seem only striated transversely.

The same authority states that in a preparation of connective tissue treated by 10 per cent. solution of potash, all the elements disappear rapidly except the elastic fibres.

When the bundles of connective tissue are treated by dilute acetic acid, as they swell they are snared at certain points by a circular fibre which surrounds them. Authorities differ as to the nature of this fibre—the so-called spiral fibre. We agree with those who believe it to be an elastic fibre. It has been objected to this view that in preparations stained by picrocarminate the fibre is coloured red, whereas it should be yellow if it were a true elastic fibre. As we believe, it is not the fibre which stains red but an amorphous plasma which surrounds it in the groove of the sheath of the bundle in which it lies. The fibre itself is obscured by this plasma and is of too small calibre to stain distinctly by picric acid. The same amorphous matter is seen stained a dark violet in gold preparations. The fibre itself can often be distinctly seen as an ordinary elastic fibre.

Elastic fibres stain in solution of magenta.

Skin.—Remove a piece of skin from any part of the body (best fresh as from an amputated limb), about $\frac{1}{4}$ inch square, with some of the subcutaneous tissue attached to it; place it

2 to 3 days in Müller's fluid, then 24 hours in alcohol. Cut it into two pieces. Embed them both so as to have transverse sections from one piece and vertical sections from the other. Stain and examine in glycerine, Canada balsam, or dammar varnish. Observe the horny layer of epidermis, the rete mucosum, the bundles of tissue which compose the corium, the subcutaneous fat, and the portions of hairs, or glands, or blood-vessels which may be included in the section.

The bloodvessels must be studied in injected preparations. The sebaceous glands can be well studied in skin from the alæ nasi or the labia majora. The sweat glands are seen in most sections of skin, but should be specially studied in the skin of the axilla, where they are unusually large. In skin from this region carefully hardened in weak solution of chromic acid for several days and afterwards in alcohol, in some subjects the layer of smooth muscular fibres can be seen on the surface of the gland coil. It is said to be present on all sweat glands. We have found it easily demonstrated only on the larger ones.

The gland cells rest on a structureless membrane which we have observed most distinctly in gold preparations. The outlines of flattened polygonal cells have been described on this membrane, but we are not aware of the nature of the method by which they were demonstrated.

The elastic fibres of the skin can be seen by most modes of preparation, but can be specially studied in the following manner: Press the skin of the anterior surface of the first phalanx of a finger firmly between the thumb and forefinger of the left hand, and with the right make vertical sections of the tense skin as thin as possible. Some of them may be sufficiently thin for use. Place them 24 hours in strong carmine, then 24 to 48 hours in 2 per cent. hydrochloric acid and examine in glycerine. The nuclei of the corium and the elastic fibres are well seen in such preparations.

Dr. Stirling gives the following method for the demonstration of cellular elements, elastic fibres, and the sheaths of the bundles in the skin :

A digesting fluid is prepared by adding to 500 CC. of $\frac{1}{2}$ per cent. solution of hydrochloric acid, a few drops of glycerine extract of the mucous membrane of the stomach, or 1 gramme of pepsine (of reliable quality). The skin to be digested is stretched over the mouth of a glass dialysing jar and placed in a beaker of the fluid, which is maintained at a temperature of 38° C. If the portion of skin is large, the fluid may be changed advantageously after two hours. The process is continued from 2 to 8 hours, according to the age of the skin and the extent of digestion which is desired. The skin is then removed and placed in water for 24 hours, when it swells and becomes transparent. Sections can be cut and stained and examined in glycerine. After prolonged digestion structures are found, which Dr. Stirling believes to be sheaths of the bundles, that have resisted the digesting process.

For the **tactile corpuscles** and the medullated nerve-fibres, the following method is recommended: From the bulb of a freshly amputated index-finger make a number of vertical sections of the thickness of a shilling or half-a-crown. Separate their deep connections with scissors and place them in $\frac{1}{2}$ per cent. solution of osmic acid. They become rapidly black. After 20 minutes to several hours begin to remove them one by one from the solution. Dry them carefully on filter-paper and embed them immediately, taking out the thinner sections first. With a very sharp knife make vertical sections as thin as possible. Make also a certain number of transverse sections. The corpuscles are seen as single, twin or triplet bodies. Each single and each member of a twin or triplet has one medullated nerve-fibre which penetrates into its interior. The fibres for the superficial members of the compound corpuscles often pass round between the members as they ascend. These facts can be made out by comparing the appearances seen in a large number of sections.

Transverse sections and various lengths of medullated fibres singly and in groups, are also seen in the corium, and fibres can often be seen lying parallel to and immediately under the rete

mucosum. Occasionally a fibre turns perpendicularly to the rete and is lost in it.

The cutting of thin sections is much easier if the skin, after being removed from the osmic acid, is placed for 24 hours in alcohol. But the best preparations are those cut without hardening by alcohol.

The same disposition of the medullated fibres can be seen in gold preparations. Place vertical sections about the thickness of half-a-crown to a crown-piece from the bulb of a fresh finger for 30 minutes in ½ per cent. solution of gold chloride. Then expose the pieces to light in distilled water made faintly sour by the addition of a few drops of acetic acid. After some days they assume a deep purple tint. They can then be embedded and cut. The medullated fibres are deeply stained, and can be traced into the substance of the corpuscles. The contour of the members of the compound corpuscles is only indistinctly indicated.

In successful preparations the finer terminal branches of the nerves in the epidermis can be seen.

Stain some of the gold vertical sections, first in carmine and then in logwood, and observe the different layers of the epidermis. A middle zone of one or two rows of cells between the rete and the horny layer (*stratum lucidum seu intermedium*) stains differently from the others.

The hairless tip of the snout of the swine is recommended as being favourable for the demonstration, by gold, of the terminal nerve-fibrils in the epidermis. The reduction may be obtained either by acidulated water, or perhaps better by the methods of Hénocque or Löwit. (See further under **Nerve.**)

Sections of the scalp are favourable for the study of the hair bulbs and the arrectores pili muscles.

The Pacinian corpuscles are sometimes seen in gold and osmic acid preparations of the skin of the finger. They are deeper than the tactile corpuscles, and near them is generally to be observed a section of a nerve bundle. In gold prepara-

tions the connective tissue of the nerve bundles is of a pearly white, and contrasts with that of the surrounding tissue.

Nails.—To see the cells, macerate in 25 per cent. solution of caustic potash, or boil for an instant in 10 per cent. soda lye.

Hairs.—Bulbs are studied in sections of skin. To isolate the cells of the hair-shaft, place it for several minutes in slightly-warm concentrated sulphuric acid, and roll under the glass. To see the cells of the medulla, macerate several days in 3 per cent. soda lye. Sections of the hair-shaft may be got by shaving, and repeating the operation shortly afterwards. The sections are found in the lather of the second shaving. (Henle.)

Fat can be examined fresh in salt solution. Tease out a fragment from the subcutaneous tissue, or spread out a small piece of omentum. To see the cell membrane, dissolve the fatty contents by maceration in alcohol or ether. To see the nucleus, stain in logwood. For the nucleus and protoplasm of the cell, as distinct from the fat, inject $\frac{1}{10}$ per cent. solution of nitrate of silver into the subcutaneous tissue immediately after the death of the animal, then cut out and examine the milky-white patch which indicates the action of the silver solution. (Ranvier.)

The special action of osmic acid on fat is seen by treating any tissue which contains it for a few minutes by a weak solution. The fat is seen as black spherical masses.

For the development of fat cells, the omentum of young animals is a convenient object of study.

Cartilage.—For hyaline cartilage, dissect out the episternal cartilage or one of the tracheo-laryngeal cartilages of the frog, and examine in water. Or, cut thin sections from the head of a frog's femur, or from the condyles of the femur of a newborn kitten or a young rabbit. Examine in water. In stained preparations, nuclei, surrounded by a granular substance, are seen.

Place a flat thin cartilage in $\frac{1}{2}$ per cent. silver solution, or rub solid caustic in stick over its surface, and examine in

glycerine. Or, rub the caustic over the head of a frog's femur, make thin sections, and examine. The dark matrix, nuclei, and a small quantity of granular substance surrounding the latter, are then seen. Between this granular matter and the ground substance, there is a small unstained zone.

This is all that can be made out by the ordinary methods of examination, and it is very little.

For anything further, a long and patient investigation is necessary.

For the guidance of those who wish to undertake the work, the following methods are recommended: Cut with a sharp razor thin slices from the head of a frog's femur, and place them in ½ per cent. silver solution for 1 to 2 minutes. Then put them for an instant in ½ per cent. salt solution. A slide is previously prepared by having a track of glycerine drawn along it from one end to the other. The sections are placed in this glycerine one by one, with the articular surface uppermost, and the whole is placed in sunlight. As soon as they become dark, examine them in succession, as they lie, with a low power, which can be done without putting on a cover-glass. Look for a section that shows a mosaic-appearance on its surface, and mount it in glycerine on another slide in the usual way for more careful examination. The mosaic is then seen to consist of darker lines enclosing somewhat square-shaped less coloured spaces, and in some of the spaces a nucleus may be seen. Although the intermediate lines are broader than the more sharply-defined lines that usually indicate an endothelium, their arrangement and the existence of a nucleus within them, shows that the mosaic in reality corresponds to a layer of cells embedded in the superficial stratum of cartilage, and their appearance and arrangement may be contrasted with that of the cellular structures usually seen in sections.

Rub the head of a frog's femur firmly with moistened solid nitrate of silver. Make sections transverse to the long axis of the bone, place them in glycerine in the manner recommended above, and expose them to sunlight. Look for sections that contain in the substance of the cartilage, deposits of black albuminate of silver in broad, somewhat circular and anastomosing bands. If, as is very possibly the case, none of these are found, the process must be repeated, and so on until it succeeds. This appearance indicates that there are spaces in cartilage in which the substance proper is wanting, and that there is an albuminous fluid in the spaces.

Make a great number of sections from the cartilage of the condyles of the femur of newborn kittens and young rabbits. Treat them with first silver and then salt solutions, and expose them in glycerine to sunlight as already directed; a considerable number, in order to save time, being placed on one slide. Search for the following appearances:

1. The surface of the sections is mostly dark, but this dark colour is occasionally interrupted by colourless patches, which may be unevenly bounded and branched. The ground substance we know to be easily saturated with silver, and the dark surface corresponds to sections of it. The uncoloured portions therefore indicate parts in which the ground substance is covered by something that acts differently in presence of the silver. That is to say, the matrix is not the homogeneous ground substance that it is generally supposed to be. Our opinion is that the colourless patches correspond to parts of delicate cellular membranes, which we believe exist in hyaline cartilage.

2. On the surface of the sections a network of broad bands of albuminate of silver may be seen. They correspond to the similar network sometimes observed in the substance of a section. Isolated patches can be observed corresponding to the position of the spaces in which the cartilage cells are usually seen, showing that an albuminous fluid is apt to accumulate in these spaces.

3. The ground substance, instead of being uniformly stained, may show dark parallel bands, about the breadth of a human red-blood corpuscle. Between the bands there are colourless spaces dividing the dark bands from each other. The straight colourless spaces are lost in unstained, somewhat irregularly-shaped patches, which correspond to the position of the ordinary cartilage cells. This indicates further that the matrix of cartilage is composed of straight uniform bands, which are separated from each other by something that does not absorb the silver solution.

In order to obtain preparations showing these three different appearances, it will probably be necessary to make a great number of sections with a very sharp razor, and both from animals newly killed, and from those which have been dead 24 hours. It is probable that some of them will be more frequently found after a slight degree of inflammation.

Perforate the cornea of an ox or a sheep and collect the aqueous humour in a capsule. Make a considerable number of sections of fresh articular cartilage (of the sheep or ox, for example), and mount them in a large drop of the aqueous humour and seal with Bruns-

wick black. (The blood serum of the frog may also be used.) Keep some of the preparations at the ordinary temperature and some at 38° centigrade. Examine them from day to day for about a week. Search on the borders of the preparation for flat hyaline cells, perfectly transparent, and without granular contents, and only to be recognised by the faint outlines of the nucleus and the border of the cell. Search further for parts in very thin sections, in which the matrix is seen to be composed of bundles which correspond to the bands indicated by the silver preparations. Larger bundles, composed of a number of the smaller bundles, can also sometimes be seen.

The preparations should be kept an indefinite time, and examined after periods of months. In one such preparation, after it was kept seven months, we found the matrix disintegrated into the amorphous nucleated masses, termed by the Germans "giant cells."

A fibrillary arrangement of cartilage is indicated, and the existence of fine bundles is also clearly shown by maceration in 10 per cent. salt solution.

The fibrillary structure is observed in the parts of human cartilage which closely adjoin the seat of diseased action.

The interfibrillary substance is in these circumstances absent, and the fibrillæ are most distinct. In sections of such cartilage, treated by osmic acid, numbers of minute oval nuclei are seen, identical in appearance with similar nuclei seen in the cornea after a brief period of inflammation. These are not the nuclei of newly-formed cells, but are nuclei which can be best demonstrated when the tissue s partly disintegrated by disease.

On the edge of sealed serum preparations fine thread-like structures are sometimes seen passing from the cellular contents of the space into the cartilage substance.

Very important results have been obtained by us by treating cartilage with saturated potash solution. Unfortunately success is in this instance very exceptional, a large proportion of the preparations being worthless. The cartilage of the head of the frog's femur is a convenient object for this treatment. The head of the bone is cut off by strong scissors and placed in the fluid entire. The cartilage is easily removed from it by needles in the form of thin pellicles, which are broken down and examined in the solution.

Successful preparations show portions of the cartilage substance isolated in layers. These layers are scooped by round or oval depressions at intervals, which correspond to the ordinary so-called cartilage cells. Layers of flat cells are found isolated, and also covering the flat portions of cartilage substance. The cells are con-

tinued into the rounded depressions in an unbroken layer, an appearance which is only reconcilable with the hypothesis that the ordinary cartilage cell or space, instead of being a closed capsule, is formed by the apposition of two layers in such a manner that the depressions in the one correspond to those in the other.

In one instance we found a portion of cartilage substance beset with minute narrow elongated cells.

The existence of layers of flat or endothelial cells in the substance of hyaline cartilage is further confirmed by silver preparations in which portions of cellular layers of various extent are indicated by the ordinary network of dark lines characteristic of endothelium. Such preparations are very rarely obtained. We have found them in sections of the condyle of the femur of young rabbits and of frogs. After inflammation of the epiphysis of the bone they are more readily seen.

For **yellow fibro-cartilage** examine the human epiglottis.

Bone.—Place a piece of fresh bone in a mixture of 5 to 8 volumes of commercial hydrochloric acid and 100 volumes of water, and let it remain in it until it is soft (several days). Place it in water to remove the excess of acid. It can then be cut by a razor. Make sections transverse and parallel to the axis of the bone, and examine them in glycerine.

Saw as thin a section as possible of a long bone (humerus or femur) from which the grease has been removed by long maceration. Grind it on a coarse glass plate with pumice stone and water by rubbing it up and down with the finger, turning it frequently. When transparent wash it thoroughly to remove every trace of the pumice stone and polish it on a smooth hone. Examine in water. The spaces in the bone called "bone corpuscles" and the Haversian canals are now visible. To see the latter lengthwise saw and polish a piece taken parallel to the long axis of the bone.

These preparations are best preserved in Canada balsam, which is hard at an ordinary temperature. A fragment of the balsam is melted on a slide, and whilst it is cooling, but before it is quite solid, the section, which must be perfectly dry, is laid on it. The cover-glass, on which a drop of the same melted balsam has been placed, is laid on immediately. The object to

be attained is that although the section is enclosed in balsam the latter shall not penetrate into the lacunæ and canaliculi. These being filled with air become conspicuous.

Very successful preparations of the osseous substance are obtained by a method which has been recommended by Ranvier. A portion of the shaft of a long bone is plunged in water immediately after having been freed from the soft parts, and before there is time for evaporation to take place. The maceration-water should be frequently changed and the bones cleaned (under water). In a year they are white as ivory and free from fatty matter. A thin section is then ground and polished as directed above. The surfaces are finally scraped with a knife to remove the paste formed by the rubbing on the stone. The section is next placed in a warm solution in alcohol of aniline blue (soluble in alcohol, insoluble in water) and then exposed to evaporation over a water-bath. When quite dry it is rubbed on a hone moistened with 2 per cent. solution of common salt. It is then washed in the salt solution and finally mounted in equal parts of the solution and glycerine. The salt solution renders the aniline quite insoluble. The Haversian canals, "bone corpuscles," or lacunæ and canaliculi are filled with aniline blue, and are seen with great distinctness.

These methods suffice to demonstrate the earthy framework of the bone and its cavities and interstices, but destroy the cellular and other structures. About the latter not much is known. For their study it is necessary to have recourse to solutions of chromic acid and picric acid. Both these acids have the property of extracting the earthy salts, whilst at the same time they preserve other constituents. Place for example a frog's femur in six ounces of a $\frac{1}{6}$ per cent. solution of chromic acid, and change the fluid daily until the bone becomes soft and flexible. Embed, cut, and stain the sections. Also place a frog's femur in a few ounces of saturated solution of picric acid, and change the solution daily. After a few days it is soft. Embed, cut, and stain. In the cavities known as bone corpuscles the nucleus of a cell is seen. Ranvier recom-

mends the following method: Place a fragment of bone at once in absolute alcohol to fix the elements, then in saturated solution of picric acid until soft, then in gum of the consistence of syrup for 24 hours, then in alcohol for 24 hours longer. Embed and make sections, which are freed from the gum by being placed 24 hours in water. Then stain.

The picric acid solution is suitable for the cells and nuclei, and the chromic acid for studying the trabeculæ in new bone. Purpurine colours the cells after chromic acid.

All that is known of a bone cell is that around the nuclei a small amount of granular matter can be stained. From this in successful preparations very fine processes can be sometimes traced to the edge of the "corpuscle" or cavity.

To decalcify adult bone it is sometimes necessary to add a few drops of nitric acid to the chromic acid solution. This should not be done until after the first few days, during which period the soft elements are fixed by the latter. In all cases it is advisable to use a large volume of solution and to change it frequently.

For the bloodvessels of bone it is necessary to use injected preparations subsequently decalcified. Red marrow is best studied by placing a fragment of a bone which contains it, such as the femur of a young rabbit or guinea-pig, in osmic acid solution for several hours, then decalcifying in picric acid and examining sections in the usual manner. Portions should also be examined fresh in serum and salt solution.

The bone corpuscles and canaliculi can be seen in a few minutes by the following method: Take the femur of a young rabbit and with strong scissors split the bone longitudinally between the condyles. With forceps detach small fragments of new bone from the edge of the medullary cavity, and examine them in ½ per cent. salt solution. The corpuscles and canaliculi are not better seen by any method. The elements of the marrow can be studied in the same preparation. Search at the same time for granular irregular masses with a number of nuclei or nuclear vacuoles. These are the so-called osteo-

klasts or myeloplâques. They should be compared with the similar structures named giant cells, which have now been found in a great number of morbid tissues. We believe that in every instance these structures are fragments of disintegrating membranous masses produced by a process of absorption which accompanies physiological or pathological changes.

To study the process of ossification decalcify the femur of young animals in chromic and picric acids. Make sections parallel to the long axis of the bone, and double stain in logwood and carmine. The new bone stains by carmine and the cartilage by logwood. The cartilage is seen to persist for a time in thin beams which project amongst the masses of new bone. The beams can be traced for a certain distance into the cartilage substance proper, whilst the extension of new bloodvessels and the absorption of cartilage take place between them.

In thin sections search for a fringe of cells on the edge of the newly-forming bone. Observe also that the canaliculi are left in the bone as it forms, and are not afterwards hollowed out of it.

The same class of sections serves for a study of the relations of the periosteum to the bone.

Von Ebner[1] has lately shown that the structure of bone is in the main identical with that of fibrillary connective tissue. Having observed that in thin polished sections made transverse to the long axis of the corpuscles, fine points are visible, and that conversely in parallel sections a fibrillated appearance is indicated, he connected the two appearances, and conceived the idea that the tissue is fibrillary, the points being transverse sections of fibrillæ. When bone is decalcified in acid, the gelatinous tissue swells and the fibrillary appearance is lost. It is therefore necessary, if the fibrillæ are to be preserved, to decalcify without causing the tissue to swell, and this he effects by the following method: It is known that strong salt solution prevents the swelling of fibrillary tissue in acids, and Von Ebner ascertained that decalcification, with preservation of the

[1] "Ueber den feineren Bau der Knochensubstanz. Sitzungsbericht der K. Akad. der Wissensch." 1875.

fibrillary structure of bone, is produced by a 10 to 15 per cent. solution of common salt, which contains from 1 to 3 per cent. hydrochloric acid. The actual mode of application of his method consists in taking a macerated bleached bone, placing it in a quantity of cold saturated solution of salt mixed with an equal quantity of water, the quantity of fluid used being in proportion to the size of the bone, and adding to it during the course of a few days successively, as much acid as will make the bone flexible. The decalcified bone is then placed for some hours in a current of water, in order to remove the excess of acid. It is next placed in cold saturated solution of salt diluted to one half. The salt solution soon becomes acid, and is then neutralised by very weak ammonia. The object remains some time (1 to 7 days, according to its size) in the salt solution, the fluid being shaken daily. It is frequently tested, and, if acid, neutralised. Large bones can only after some time and with trouble be got neutral; but with small bones this result is obtained in a few days. When neutral, the bone is not transparent like the so-called "bone cartilage" produced by the ordinary action of acids, but is white and non-transparent like bone which has not been decalcified.

Through the bone, thus prepared for examination, sections are made, parallel and perpendicular to the long axis, and in various oblique directions, and examined in water. If the bone is not quite neutral, they are examined in 10 per cent. salt solution. Parallel sections are also scraped with a scalpel, and the substance thus removed is spread out and examined. The fibrillæ which are thus isolated, present under the action of acids and alkalies all the properties of ordinary fibrillary tissue. The results of his investigations by this and other methods, for the details of which reference must be made to his memoir, are brought together by Von Ebner in the following sentences: "I. The ground substance of bone is composed of gelatinous non-calcified fibrillæ, which are held together by a cement substance in which the earthy matters are contained. II. The bone-fibrillæ form bundles of about 3μ diameter. These bundles, in a single layer or several layers over each other, are connected by numerous anastomosing fibrillæ which are given off at an acute angle, and thus form a closely-interwoven plate, designated a primary lamella. If several primary lamellæ follow each other with a similar direction of the fibres, secondary lamellæ of varying thickness are formed, the lamellar arrangement being both in cut and in ground sections so much the more conspicuous, the more the direction of the fibres changes in consecutive layers. III. The bones of the human adult are built up of a number of small portions, which are of mani-

fold shape and bounded by uneven irregular surfaces. They hang together only partially by means of bundles of the bone fibrillæ; for the most part, however, they are completely independent of each other, and are only bound together by a firm cement. These portions may be limited on all sides by others, which are the seat of absorption of previously-formed bone, or of apposition of newly-formed bone, or partly by absorption and partly by apposition surfaces. The question of the beginning and end of the bone lamellæ is thus solved. They are limited either by a gradual transition into the irregular fibrous tissue of an apposition-surface or by the sharply cut out border of a surface which is undergoing absorption. IV. The fibrillæ may be without definite order (as on the apposition surfaces in the cement lines) or arranged in bundles. The bundles may vary considerably in thickness. The finer bundles may form lamellæ, or simply a tissue with arrangement of parallel fibres. The coarser bundles never form lamellæ, but may form a special variety of bone tissue by interweaving in different directions. There are thus, in all, three kinds of bony tissue: 1, felted bony tissue; 2, bony tissue with parallel fibres; 3, lamellar bony tissue."

We have obtained preparations showing the bundles which compose the bony tissue, by the following method: Portions of the femur of a frog are placed in ¼ per cent. solution of osmic acid for 1 to 2 days, then decalcified by picric acid. In thin longitudinal sections, parallel bundles, having a breadth nearly equal to that of a human red blood corpuscle, can be sometimes distinctly seen.

Sharpey's fibres consist of isolated bundles of bony tissue which penetrate the bone from the periosteum. They are seen when the lamellæ are torn asunder.

Teeth, like bone, must be ground or decalcified. It is not practicable to make sections with a saw. Exner gives the following method: Drop melted wax on the end of a cork until the tooth can be fastened in it. Then add more wax until the tooth is completely covered. Grind wax and tooth very carefully on an ordinary grinding-stone which is turned with a handle. When a smooth surface has been obtained soften the wax and remove the tooth. Embed it a second time with the ground surface downwards and grind as before until the wax can be seen through the tooth substance. Then finish with pumice-stone and a hone as for a section of bone.

The methods used in the study of bone are applicable to that of the teeth. This is also the case with Von Ebner's method.

Striped Muscle. — The breast muscles or thin muscles attached to the lower jaw of the frog may be conveniently selected for examination in $\frac{1}{2}$ per cent. salt solution to show the fibres and the striation.

To see the sarcolemma treat fresh muscular fibres with water. To see some of the nuclei connected with the sarcolemma treat fresh muscle with dilute acetic acid.

For a further study of the structure of the fibres the following methods will be found useful: Dissect the skin from the leg of a frog and cut the femur across at its middle. Place the separated part of the limb in a pint of $\frac{1}{6}$ per cent. solution of chromic acid. Change the solution frequently for 3 weeks. Embed the fleshy part of the gastrocnemius and make transverse sections, taking care that the knife is very sharp. Float the thinnest of the sections direct from the knife to a slide, draw a current of water over them by filter-paper, add a drop of logwood solution until staining takes place (as observed under a low power), wash by another current of water, then add a drop of glycerine, put on a cover-glass and examine. These operations must be so carefully and gently done that the section is not moved nor injured. After the cover-glass is applied the glycerine, which is necessarily diluted with water, should be replaced by drawing through pure glycerine.

In such a section each muscular fibre is seen to be surrounded by a ring of delicate connective tissue. The rings communicate and form a network. There is a clear space between the mesh and the muscular fibre. In the fibre itself there is to be seen the sarcolemma in section and within the sarcolemma sections of the fibrillæ. (In parts of such sections we have been able to distinguish fine groups of fibrillæ, each group corresponding to one of Cohnheim's fields.)

Tease out very gently some of the thicker sections and examine them in glycerine. The parts of the fibrillæ removed in

the section can then be seen lengthwise. It can be observed that each fibrilla is a fine cylindrical element, and that all the fibrillæ have the same thickness.

Place a leg of a frog, similarly prepared, fresh, in equal parts of water and alcohol for several days. Cut sections of muscle and examine the fibrillæ.

Place a small portion of the gastrocnemius of a frog in ½ per cent. gold solution for 20 to 30 minutes. Then expose to light for several days in water acidulated with acetic acid. Tease out the individual fibres carefully in glycerine. Examine the fibrillæ. It will be observed that they adhere to each other with considerable tenacity, and that it is only at some parts that an isolated individual fibrilla can be seen. Adherent to the fibrillæ is a greater or less quantity of a dark substance, which is especially marked at points corresponding to the transverse striation. Even on the isolated fibrillæ this substance is to be seen.

Compare the results of the treatment by chromic acid, diluted alcohol, and gold with the object of determining whether the fibrilla is a definite structural unit or whether there is a possibility of fibrillation in muscle to an indefinite extent.

We believe that the fibrilla is a definite element, which is isolable by various methods, and that it is not a result of fibrillation produced by reagents. The figures of fibrillæ in text-books are mostly representations of a number of fibrillæ closely adherent to each other.

Make transverse sections of frozen muscle and examine them in ½ per cent. salt solution. Observe that the section of a fibre is made up of minute structures mostly with 3 and 4, but sometimes with 5, sides. Cohnheim was the first to describe this appearance, and the elements are named "Cohnheim's fields."

We have observed them satisfactorily by the following method: Fresh muscle is cut transversely with sharp scissors, the thin pieces so excised placed in a strong solution of extract

of logwood and alum, and the excess of colouring removed by acetic acid.

Ranvier ("Traité technique") states that they can be seen after the action of ½ per cent. solution of alum and of alcohol diluted to a third, and also by the following method: Lay bare the sartorius of a frog and inject 1 per cent. solution of osmic acid into its substance. Remove the muscle and place it in alcohol. Place it afterwards in solution of gum and again in alcohol to harden the gum. Then cut transverse sections which are freed from gum in water and examined in glycerine.

Place fresh muscular fibres in 1 per cent. acetic acid or ½ to $\frac{1}{10}$ per cent. hydrochloric acid. After some time it will be found that the longitudinal marking has disappeared, and that the transverse marking is unusually distinct. It will be also seen that the fibre has a tendency to cleave into transverse discs, the cleavage taking place in a line with the transverse marking. The cleavage takes place through the whole thickness of the fibre.

Place fresh fibres in concentrated solution of carmine until they are saturated with the dye. Then place them in diluted acetic acid until they are pale in colour and examine in glycerine. The fibres are seen to be snared by fine thread-like filaments (spiral fibres), and bulge between the snared points.

As Cohnheim's fields are invariably found in properly prepared muscular fibre, it necessarily follows that the fibre is constituted by a number of parallel cylindrical structures enclosed by the sarcolemma. These structures are seen imperfectly by many of the usual methods, the spaces between them being indicated by lines of minute granules, which are described by some authors as protoplasm—an unsupported assumption regarding their nature. The structures themselves were described by Leydig as *primitive cylinders*. We have described them as *primary bundles*, in order to indicate that they are analogous in general structure to similar elements in connective tissue, and that they do not represent the ultimate formed unit, which we

believe to be represented by the fibrilla, each element being a bundle of fibrillæ.

These primitive cylinders or bundles are capable of demonstration. Ranvier gives the following method: Remove the thigh of a frog with part of the pelvis and tibia attached (so as to preserve the muscles in extension) and place it for several minutes in $\frac{1}{5}$ per cent. solution of osmic acid. Then place it in water and detach some of the superficial fibres. After being 24 hours in 1 per cent. solution of picrocarminate place them on a slide in a drop of pure acetic acid, and press them with a cover-glass. Substitute glycerine for the acetic acid and the preparation is permanent.

Remove a bundle of muscular fibres of the wing of the water-beetle and place it on a slide, isolate the fibres as quickly as possible with very fine needles and without adding any liquid. The *half-drying* process which they thus undergo facilitates the extension of the fibres. To prevent their becoming quite dry it is sufficient to keep them moist by breathing on them. Place on the fibres several drops of solution of hematoxylin (Bœhmer's). In a few minutes, when they are coloured, remove the dye by drawing water over them (without disturbing the fibres). Repeat this successively with absolute alcohol and oil of cloves and finally with Canada balsam, in which they are permanent. The muscular fibre is found not to be equally stained throughout. In favourable preparations and with a high power the following appearances are to be seen: Broad coloured bands and unstained bands alternate with each other. Further, a faintly coloured line divides the unstained band transversely into two portions, and the broad stained band is also divided into two portions transversely by a central faintly stained part: the former corresponding to the ordinary transverse marking, the latter to Hensen's stripe (Ranvier, *l.c.*). We have observed the same appearances by the following simple methods: Place the leg of a crab entire in equal parts of methylated alcohol and water for from 24 to 48 hours. Then break it and excise a portion of muscle, tease it out, stain

in logwood, and examine in glycerine. The alternation of the stained and unstained bands is very distinct. On account of the action of the alcohol it is easy to tease these fibres into longitudinal fragments in which the individual fibrillæ and the bundles or cylinders can be sometimes observed. The alternation of staining can be seen on these fragments, even the smallest of them.

In order to observe what it is that stains, we recommend a series of preparations to be made as follows: Wrench off several legs from a crab, and place them in a small covered capsule without the addition of any fluid. Begin after a few hours to open them, and tease the fibres in glycerine strongly tinged with picrocarminate. Repeat the process on others at intervals of 1, 2, and 3 days. Tease out and examine. Amongst the preparations so made, the following appearances are to be found. In fibres in which the alternate staining exists, the minute fibrillæ can be traced unbroken through the stained and unstained portions. In the stained bands, the coloured matter is observed to be diffused amongst the fibrillæ, and faintly stained fibrillæ can sometimes be observed in a much more deeply stained medium. In other preparations the stained formless substance is continued indefinitely through the fibre: the fibrillæ can be seen in it, but all appearance of alternate stained and unstained bands has vanished.

Treat some of the preparations in which the alternate stained bands are well seen, and in which the staining has been intense, by acetic acid, and search for parts in which transverse cleavage has taken place. It will be then observed that the cleavage takes place between the stained bands.

The conclusion we draw from these stained preparations is, that the substance in a muscular fibre which colours in picrocarminate and logwood, is not identical with the fibrillæ, and that it is frequently found accumulated in distinctly demarcated portions of the constituent parts of the muscle, the intermediate parts being free from it. It is possible that this condition is constant in living muscle. In our opinion, it does not show

that the primitive cylinder or bundle is made up of discs of different substances interpolated between each other — an hypothesis that has been formed to explain this and other appearances.

In some of the picrocarminate preparations prepared in this way, we have observed in the substance of the fibre a number of minute oval nuclei, very much smaller than the other nuclei which can by certain methods be observed in muscular fibre.

To observe the phenomenon of muscular contraction, tear out the leg of a water beetle, cut the carapace of the largest joint, remove the muscle quickly and carefully, and examine it in the drop of fluid which oozes from the wound (Ranvier) or in white of egg (Merkel). To prevent evaporation, seal the preparation in paraffin.

The fresh muscular fibres of the common crab found on our coasts form a beautiful and instructive object of study, and especially for the observation of the transverse markings. These can be traced in direct parallel lines across the fibres, the stripe from one fibre being continued optically unbroken into that of its fellow—an observation that ill accords with the supposition that the striæ correspond to separate minute discs in each individual primary cylinder or bundle of a fibre.

Ranvier (*l.c.*) has lately studied the conditions of contraction by injecting osmic acid into the muscle of a rabbit: 1, relaxed by the position of the limb; 2, extended; and 3, by exciting contraction in an extended muscle. This is done by fixing an electrode in one end of the muscle, the other electrode being in the syringe. Thirty to forty shocks a second are given, and the osmic acid is then injected. The conclusion drawn from the appearances observed is, that contraction is attended by a change in the relative position of some of those constituent parts of the fibre, which produce the appearance of alternating discs.

There are other facts of importance regarding the structure of muscle which we have described in detail elsewhere. The methods by which they were obtained are successful in exceptional instances, and we can only recommend those to repeat them who are willing to devote to the subject the time and labour required for a long in-

vestigation, and who are not to be discouraged by repeated failures.

Cut transverse portions of the fresh gastrocnemius of a frog, place them 20 to 40 minutes in ½ per cent. gold solution, and expose them to sunlight in acidulated water, until they are dark in colour. Search for the following appearances :

1. The transverse section of a fibre shows the muscle substance contracted towards the sarcolemma, producing the appearance of a central canal.

2. In teased fibres, search amongst the fibrillæ for flattened narrow elongated cellular elements, lying parallel to the long axis of the fibre, and for rounded or oblong flattened cellular elements.

3. In transverse section the surface is sometimes seen divided into compartments by a dark network, with central nodal points in which nuclei may be observed. (Each of these compartments corresponds to an area which would contain a considerable number of Cohnheim's fields. We have designated them secondary bundles.)

Macerate portions of fibres stained by gold in the manner just indicated, in the ordinary concentrated acetic acid of commerce for periods varying from 6 to 24 hours, and place some of them with the acid in test tubes, which are kept in a water-bath for a similar period at a temperature of 100° to 110° Fahr. Tease out the fibres carefully, and search for isolated portions of a network of very fine fibres with narrow meshes. Intercalated at several points of the network are small masses of cellular substance deeply stained by the gold. A portion of such a network is shown in fig. 4.

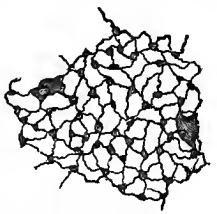

Fig. 4. Network isolated from muscular fibre of frog.

By mixing ½ per cent. solution of chloride of gold with acetic acid in varying proportions, and sealing fresh muscular fibres in the mixture on a slide by Brunswick black or asphalt, a combined action is produced which throws light on the structure of the fibre. In some preparations the fluids should be in equal parts, and in some the acetic acid should be in excess. The preparations should be examined frequently over a period of days. The following appearances are to be looked for :

1. The substance of the muscular fibre is very transparent, and in the transparent medium a number of delicate fibres with elongated oval swellings can be seen. They are parallel to each other,

and can be occasionally seen to project beyond the edge of the muscle substance. This appearance is shown in fig. 5.

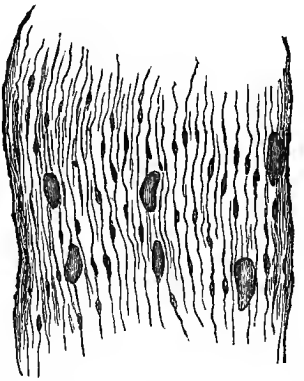

Fig. 5. Muscular fibre of frog sealed in a mixture of gold solution and acetic acid.

2. The muscle substance is extruded from the fibre proper in the form of minute discs, which are seen singly and in groups. We have observed these discs in the muscular fibre of the mouse. They are about the size of a human red blood corpuscle. The appearance highly magnified is shown in fig. 6.

The groups in this figure indicate, in our opinion, the larger compartments in the fibre which we have described as secondary bundles, and we have advanced as a reasonable hypothesis the theory that the discs are produced by the acetic acid causing the bundles to swell against the fibres of the network—the increased consistence which renders this possible being the effect of the gold solution.

In a muscular fibre of the mouse, treated first by chloride of gold and then sealed on a slide in dilute acetic acid, we found, after 12 months, that one part of the fibre showed the transverse markings as hoop-like rings surrounding the fibre, whilst at another there was no appearance of markings or rings, but bulging fibrillæ, spreading out as if unconfined by any constricting element. The whole appearance might be aptly compared to that of a birch broom.

In fibres of the frog which had been stained by logwood and preserved over a long period in glycerine, we found a perfect demon-

stration of isolable primary bundles or cylinders, of narrow elongated cells investing them, of oblong, flattened cells covering the secondary bundles, and of large nuclei on the sarcolemma.[1]

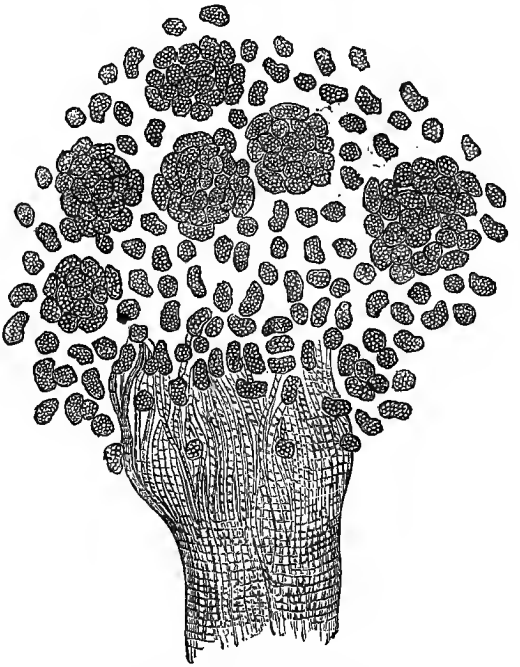

Fig. 6. Muscular fibre of mouse. Discs of primary bundles of fibre produced by combined action of gold and acetic acid.

Muscular fibre may also be advantageously studied by the action of saturated solution of caustic potash. In the fibres of frog's muscle, treated by this method, appearances indicative of one, and sometimes two central canals, filled with nuclei, are seen, and correspond to similar appearances figured by Amici in a memoir, translated in the 16th volume of Virchow's "Archiv." (1859). It does

[1] For further details regarding this observation and the methods, we refer to a memoir on "The Structure of Muscular Fibre," in the *Quart. Journ. of Mic. Science* for 1876.

not follow that these are closed canals. They are probably large spaces between the bundles, the cellular structures belonging to the investing cells of the latter. By maintaining the temperature of the potash over a period of hours, instructive stages in the disintegration it causes are to be observed. Amongst these are oval, clean-cut holes in the sarcolemma, which may correspond to the position of the "end-plates" to which the medullated nerve fibres can be traced. It is through these holes that nerve fibrils probably pass into the interior of the fibre. They also afford a ready means of communication between the parts external and internal to the sarcolemma, and allow a free passage for the lymph.

When fresh muscular fibres are treated by weak acids, aud swell slowly, the sides of the fibre in optical section show a regular series of bulgings and constrictions. The constrictions correspond to the transverse stripes, and prove that there is a substance in these lines that resists swelling in acids, and that exercises pressure on the swelling muscle substance. It is to be remembered that it is in the same lines that transverse cleavage takes place. These facts, taken in conjunction with the isolation of a transverse network, have led us to suggest the network as a cause of the transverse stripe or line, and its cutting action in swelling muscle as the cause of transverse cleavage.

This explanation does not, of course, apply to the intermediary disc of Hensen, seen by various modes of preparation, which crosses the bundles or cylinders between the ordinary transverse stripes. It probably depends on a peculiar disposition of the interfibrillary substance.

If the leg of a water-beetle is torn out of its socket, the chitinous case quickly laid open, the muscle removed with scissors, and, after being rapidly teased on a slide with needles, examined under the microscope, waves of contraction may be observed passing along some of the fibres.

An examination of fresh muscular fibres may be made in the same preparations. The study is very interesting, but with our present instruments is not fitted to throw much light on the elementary structure. Indeed, the latest observers by this method do not seem to have had results so successful as those published by Amici (*l.c.*) nearly twenty years ago.

We have observed an appearance in some of the fibres from the crab's leg, treated by the above methods, which has been noticed before and is worth noting. The fibrillæ formed parallel zigzags in the fibre, the angle of the zigzag corresponding to the transverse

stripe. It is clear that this condition might be connected with a shortening and swelling of the fibre, and the fibres in which it is found may possibly have been arrested in contraction.

It is possible to show colourless silver fields in the substance of the muscular fibres of the frog (Cohnheim). For this Gerlach recommends the following method: Prepare four watch-glasses, the first with $\frac{1}{2}$ per cent. salt solution, the second with distilled water, the third with $\frac{1}{10}$ per cent. solution of nitrate of silver, and the fourth again with distilled water. A few drops of the salt solution are placed on a cover-glass, in which muscular fibres from a newly-killed frog are isolated as completely as possible under a dissection lens. After they are thus prepared they are allowed to remain in contact with the salt solution for 8 to 10 minutes. They are then quickly drawn through the distilled water of the second watch-glass to remove the solution which is external to the fibres, but not to remove that which has penetrated into their substance. They are next placed in the third watch-glass (the $\frac{1}{10}$ per cent. silver solution), and remain in it until they assume a dull white colour, which is generally the case in half a minute. The fibres are then carefully washed in the fourth watch-glass, and finally exposed to direct sunlight from 5 to 10 minutes, until they are of a reddish-brown colour. They are next placed for 3 to 4 minutes in $\frac{1}{10}$ per cent. solution of hydrochloric acid, in which fluid they are again carefully isolated, and are then ready for examination. They can be preserved in glycerine or, after treatment with alcohol, in Canada balsam. The "fields" in the substance of the fibre thus obtained are in successful preparations elongated colourless bands, sometimes of considerable length. They communicate with each other by anastomoses. Their interpretation will depend on the views held regarding the structure of muscular fibre. According to our opinions on that question, they correspond to interfascicular spaces.

The **union of muscle and tendon** may be studied by two methods. If a thin muscle, ending in a straight tendon, is placed for some hours in $\frac{1}{2}$ per cent. osmic acid and the preparation is carefully teased out in glycerine, it is possible to trace the constituent parts of some of the muscular fibres in direct continuity with the tendinous structures. That the continuity is not simply one of the sarcolemma is shown by its being observed to hold good for parts of the fibre. In muscular fibres which meet their tendon obliquely or which end in an aponeurosis, this is less evident, but even here the conical end of the fibre can be traced into a purely fibrous tissue, which is of much smaller calibre than the fibre. But if, on the other hand, a muscle

with its tendon is placed in 40 per cent. solution of potash, the fibres retract from the tendon, the conical end of each fibre corresponding a concave surface of tendinous substance. In order to reconcile this observation with that of the osmic acid preparations, we must either suppose that the union between the muscle and tendon, seen in the latter, is only an apparent one, or that it is formed by some substance which is neither muscle nor tendon, or that there is a force in action at the point of junction which is not in force at any other part of the tendon. The two former suppositions are not supported by any known fact, and they are not in accordance with the direct continuity observed in well-teased-out osmic acid preparations. We therefore prefer to adopt the last of them, and to believe that changes produced in the muscle substance by the strong alkali cause the displacement which is observed. It may well be that, although there is a continuity of fibrillæ, the fibrillæ in the muscle substance are less resisting than those which are found in tendon.

Involuntary Muscle.—Convenient objects for the isolation of the cells are the stomach, intestine or bladder of the frog, or intestine or uterus of mammalia. Maceration for from 24 to 72 hours in 20 per cent. nitric acid, or from 15 to 30 minutes in 35 per cent. potash solution.

By treatment with saturated potash solution they can be seen instantaneously. They can also be isolated in weak solutions of chromic acid and bichromate of potash.

They can be seen in optical section in small arteries in gold preparations of the omentum.

To see their arrangement in bundles, freeze and examine the sections in iodised serum.

To show that the cells are fibrillated, Ranvier recommends the distension of a piece of intestine with alcohol. It is secured by loops and placed further in alcohol for 2 days. The muscular coat is stripped off in fragments by forceps, stained and examined.

In the fresh tissue, treated by silver solution, the outlines of the cells are indicated by dark lines.

Krause states that the cells can be isolated by maceration

for several days in 10 per cent. salt solution. He states also that transverse markings can be observed in fibres from the œsophagus of man examined in water several hours after death.

Nerve.—To see medullated nerve fibres, cut out a piece of the sciatic nerve of a newly-killed frog, place it immediately on a slide in a drop of blood serum, and cut it against the slide into longitudinal fragments with a sharp knife. Cover and examine.

Instead of simple serum, iodised serum may be used, and the preparation at once sealed by Brunswick black. It can then be examined at leisure. If carefully put up, the fibres remain for one or two days scarcely altered.

The coagulation of the myeline, which usually begins to show itself almost as soon as the fibres are placed on the cover-glass, can be accelerated by adding a little water.

Lay bare the sciatic nerve of the frog, and let fall on it immediately some drops of osmic acid solution. Cut out a portion and place it for 10 or 20 minutes longer in a few drops of the same solution. Divide it into several pieces. Tease out one in glycerine. Stain others in picrocarminate, aniline, and log-wood respectively. Tease out and examine in glycerine. Observe the medullated fibre stained black, the remains of the delicate connective tissue sheath around each fibre where it persists, and nuclei. Look for fibres in which there is a constriction or snaring—Ranvier's nodes. This appearance may be seen in every peripheral nerve fibre after certain modes of preparation.

Seal a portion of a nerve in blood serum, and examine it after some hours, and over a period of several days. In some of the fibres a transverse hyaline line may be observed. It corresponds to Ranvier's nodes.

The Ranvier nodes as seen in fibres treated by osmic acid, and as they are generally described, consist of a series of breaks or

interruptions in the medulla, which divide the nerve fibre into segments. At the ends of the segments the medulla suddenly tapers, and a distinct space, unoccupied by medulla, is left between the segments. In this apparently non-medullated space, the axis cylinder is often seen very distinctly. In the sealed serum preparations, on the other hand, the medulla is unbroken throughout, undergoing no constriction, and the straight fine hyaline line which crosses it does not seem to be connected with any displacement. That the condition seen in the serum preparations corresponds to the condition in life, and that the constriction and separation of the medulla of the different segments is artificial, may be shown by the following method : Pith a frog and dissect the skin from one of the legs. Pass a ligature firmly, but not with much tightness, round the thigh, near the head of the femur, and another near the knee. Separate the thigh above the upper ligature and below the lower. Cut off those muscles that are not in immediate contact with the sciatic nerve : the nerve itself must not be exposed. Place the whole in $\frac{1}{10}$ per cent. solution of osmic acid for 24 hours. Then dissect out the nerve. The part at the cut ends will be found black, and the central part perhaps scarcely coloured at all. Tease out and examine in nearly saturated solution of acetate of potash an intermediate portion in which the action of the acid has been moderate and gradual, and find fibres in which the nodes are visible. It will then be observed that they are indicated by a fine straight line which crosses the fibre, the appearance seen in the sealed serum preparations being exactly reproduced. There is no depression or constriction of the medulla at the extremities of the line, the borders of the segments accurately fitting each other. The appearance is the same as if a cylinder had been evenly cut through and the two ends accurately apposed again to each other. It seems, therefore, as if the exaggerated appearance of the nodes which is usually seen in osmic preparations, is produced by a retraction of the medulla under the influence of the reagent.

We have seen an exactly similar condition of the nodes in carefully manipulated gold and formic preparations, in this case the straight transverse line being indicated by a minute quantity of gold deposit, and no constriction breaking the straight lateral contour of the medulla.

That this appearance is due to a structural peculiarity is beyond doubt ; but we are of opinion that the exact nature of this peculiarity is not known. It may possibly consist in a division of the medulla into segments, but the hypothesis is not excluded that it is produced *post mortem* by the action on the medulla of histological

elements immediately external to it; possibly minute circular fibres connected with the primitive sheath (sheath of Schwann), and analogous in their effects to that of the spiral fibre which constricts the more persistent bundles of connective tissue.

In sealed serum preparations of the sciatic nerve of the frog there may sometimes be detected within the first 48 hours detached portions of endothelial layers. The layers are usually double, and the nucleus and cell substance are distinct. They belong to the perineurium, and resemble the similar layers found in like conditions in preparations of muscle and tendon.

Place a portion of a nerve for 24 hours in absolute alcohol. Tease it out in solution of logwood. Stain and replace the dye by glycerine. Observe the nuclei (in exceptional instances very numerous) and the axis cylinder, which is sometimes stained.

Place a portion of a nerve in purpurine solution for 24 hours. Tease out and examine. The axis cylinder may be seen.

In transverse sections of hardened nerves, stained in carmine, the axis cylinder is distinct.

A transverse section of the sciatic nerve of a dog is useful to demonstrate the connective tissue sheaths around the smaller and larger bundles that compose the entire nerve.

Aniline red stains the axis cylinder.

Collodion added to a fresh nerve teased out on a glass without reagents also demonstrates it.

One of the most important reagents we possess for studying nerves is chloride of gold, but the conditions of its successful application are so various that it may be said to have a special *technique* of its own. These conditions may be learnt by applying it to the nerves of the cornea.

Cut out several corneas from the eyes of frogs, guinea-pigs, and mice within a few hours after the death of the animal. Place them in ½ per cent. gold solution for 20 to 25 minutes, and then expose them to light in water slightly acidulated by acetic acid until they have acquired a violet colour. Examine them in glycerine. The extent to which the nerves are visible will be found to vary much in the different corneas. In some

of them the nerves will not be visible at all. It is now, on the other hand, well established that a similar treatment after a few hours' inflammation will infallibly produce a demonstration of the most abundant nerve fibrillæ of the finest order. The latter consist of a network formed by extremely delicate beaded threads, and that these are nerve structures is shown by their continuity with the nerve fibres that enter the cornea at the limbus.

The demonstration of the nerves in the superficial laminæ of the healthy cornea of the rabbit is by no means certain in any given instance; but we have found, in studying rabbit's corneas, which had been the seat of congestion for a few hours, that the finest fibrillæ were seen in great numbers.

Similarly, in the normal epithelium of the anterior surface of the cornea the terminal network (we can testify to the existence of such a network, as distinguished from the so-called terminal bulbs) is difficult to get by the gold method unless there has been slight and temporary congestion previously.

It is finally to be noted that the demonstration of the nerves by the gold method succeeds more easily in the cornea than in any other structure.

The explanation of this is, not that there is anything in the nerves of the cornea by themselves peculiarly suitable to the action of gold chloride, but that the consistence of the cornea is sufficient to keep the nerves in their natural position and uninjured whilst the solution is acting on them. The demonstration of the nerves succeeds better when the cornea is inflamed, because the spaces between the corneal laminæ and bundles are distended and the solution can easily pass into them.

It is on this principle that various successful methods have been applied to the demonstration of the nerves in different organs, and it is by keeping it in mind that it will be possible to invent others which will enable us to trace the nerve distribution in structures in which it has not yet been followed. The solution should be brought into contact with the nerves

whilst the tissues are in their natural position; tension, twisting, collapse, or pressure being equally avoided. Fortunately, the after manipulation is assisted by the increased consistence which the gold solution gives to yielding structures.

The strength of the dilute acetic acid is usually determined by the taste, the acid being added in drops until the fluid is distinctly but faintly sour. This corresponds to 1 to 2 per cent. solution of the ordinary commercial acid. The astringent effect of the gold is sufficient to neutralise the action of the acid until the nerves are demonstrated. Whilst exposure to light is taking place the fluid should be changed occasionally, the reduction of the gold taking place within 2 days to a week. In some tissues, such as a large cornea or a portion of skin, the gold solution is allowed to act from half an hour to an hour and a half.

This is the method originally suggested by Cohnheim. Various modifications have been since introduced. For the cornea of the rabbit or guinea-pig, Dr. Klein recommends exposure to light in simple distilled water after a long action of the gold solution (1½ to 2 hours). The cornea is washed in distilled water after being removed from the solution, and the water in which it is placed is changed twice or oftener during 24 to 36 hours. It is then placed for 2 or 3 days in a mixture of one part glycerine in two of water. After the gold precipitates are brushed from the surface, sections can be made.

Hénocque has pointed out that if a tissue treated with gold is placed in a concentrated solution of tartaric acid, and the tube containing it is heated in a water-bath, the reduction of the gold takes place with great rapidity.

The following methods by Löwit illustrate the principle on which successful gold preparations are obtainable. In an investigation of the nature of the terminal nerve fibrils in involuntary muscle, he proceeded thus: The bladder of a frog is first distended for 5 to 8 minutes with a weak gold solution in order to harden it sufficiently so that it will not shrink in

stronger solutions. The bladder is then placed in a very weak solution of acetic acid (2 to 3 drops of ordinary acid in a watch-glassful of water), and left in it until the epithelium can be easily removed by a brush. It is then placed in a 1½ per cent. gold solution for 5 to 8 minutes, and after being washed in water is exposed to light in water made strongly sour by acetic acid. In 24 hours the sour water is changed and the acidity increased. By this method some excellent preparations were obtained, but in many cases it failed. The following method was therefore suggested by the same observer. Pure formic acid is selected of a specific gravity 1·12, stress being laid on the purity and on the right specific gravity. The bladder is first distended with weak gold solution as before, and then placed in the undiluted formic acid for 3 to 5 minutes. It is next transferred directly to 1½ per cent. gold solution, in which it remains 5 to 8 minutes. After being washed in water it is then placed in a stoppered bottle containing a mixture of one part of the acid and three parts water; 8 to 10 C.C. of the mixed fluid being a sufficient quantity for the frog's bladder. The bottle is kept for 24 hours in the dark. For thin objects this time is sufficient for the complete reduction of the gold. If a preliminary examination in water shows that this has not taken place, the object can be placed for another 24 hours in a mixture of equal parts of the acid and water.

Before being mounted in glycerine the preparation must be thoroughly washed in water in order to remove the excess of acid.

Thicker objects, such as intestine and cornea, were placed fresh in the undiluted formic acid 10 to 15 minutes, then 30 to 45 minutes in 1½ gold solution, and, after being well washed, placed in the dark, for the first 24 hours in a third acid with two thirds water, and for a second day in equal parts of acid and water. The tissue can then be hardened in alcohol, and embedded and finally preserved in dammar.

The following methods for the demonstration of the nerves of

muscular fibre are extracted from a monograph by Gerlach,[1] which may be further consulted.

The first was suggested by Kühne. Portions of the sartorius of the frog, as long as possible and freed from tendon, are placed in a solution of sulphuric acid, prepared by adding 0·1 gramme of acid of sp. 1·83 to a litre of water. The vessel is shaken and allowed to stand 24 hours, during which period the diluted acid is changed twice or thrice. The muscle is then taken out and washed by shaking it in a test tube with distilled water until the fluid no longer reddens litmus paper. It is then placed in a large glass vessel filled with distilled water, and exposed in an incubator for 22 hours to a temperature of 35° to 40° C. The muscle, now become transparent, is shaken in a test tube with water and falls into single fibres. The entrance of the nerve into the fibre within the sarcolemma and the first intravaginal division of the axis cylinder may be observed.

Another method consists in teasing out muscular fibres from a newly-killed frog in $\frac{1}{15}$ per cent. hydrochloric acid. A cover-glass is put on, and from time to time more of the fluid is added. After some hours the muscle substance becomes transparent and the intravaginal nerves are visible. By this treatment in many fibres the transverse striation disappears, and if the action is allowed to continue for a day, parallel equidistant longitudinal markings appear. Gerlach remarks that the relation of these lines to the structure of the fibre is involved in complete obscurity. We identify them as shown in his figure as the boundary lines of primary bundles.

Rollett's method for isolating muscular fibres may be applied to osmic acid preparations thus: A thin muscle is placed for 4 or 5 minutes in weak osmic acid. After being boiled it becomes rapidly dark in colour, and is then placed for several days in glycerine. It is next placed in a test tube half full of glycerine, the tube being kept corked and shaken several times daily. After 5 or 6 days the isolation is usually complete, and some of the nerves are preserved. More nerves are preserved when the muscle is treated with $\frac{1}{2}$ per cent. hydrochloric acid for several days, before being placed in the glycerine.

The very important results described by Gerlach were obtained by means of the following modification of the gold method: One part of the double salt, the chloride of gold and potassium, is dissolved in 10,000 parts water, and one part of chemically pure hydro-

[1] "Das Verhältniss der Nerven zu den willkürlichen Muskeln der Wirbelthiere," von Dr. J. Gerlach. Leipzig, 1874.

chloric acid is added to the solution. This constitutes his "gold fluid." The muscle must not be placed in the fluid immediately after death, nor must *rigor mortis* have begun. In the frog he found from six to nine hours after death the most suitable time. A state of tetanus in the muscle is favourable to the reaction, and in this case the fluid should be applied three or four hours after death. Tetanus results when a frog is killed by hitting its head sharply several times on the edge of a table. After the violent muscular spasms, which last several minutes, have abated, the lower extremities are quickly removed from the trunk, in order to prevent the condition of the muscle being changed by the influence of the circulation. Long fasciculi from the gastrocnemius are placed in the fluid, and the fibres teased out in it into fine bundles, although not into completely isolated fibres. They remain in the gold fluid for 10 to 12 hours, protected from light. After this time they are treated with faintly sour water, and if the preparation has been successful a lilac-reddish colour is seen at some points. When exposed to light in glycerine slightly acidified by hydrochloric acid, the intravaginal network is completely developed.

In other less successful preparations the whole fibre is of a diffuse red colour, and the nerve fibrillæ are not seen. If such preparations are placed 5 to 6 hours in the acidified glycerine a further reduction of the gold salt takes place. If they are then treated with ½ per cent. solution of cyanide of potassium the colour gradually disappears, but at some portions the intravaginal network retains it for a time, and is thus rendered visible. At any given stage the action of the cyanide may be arrested by washing the preparation carefully with water, and it may then be preserved in gum and glycerine.

By this method Gerlach has demonstrated that the so-called end-plates do not represent the terminations of the nerves of voluntary muscle. The medullated fibre, after dividing, passes through the sarcolemma into the interior of the fibre, where it immediately loses its medulla, and long axis cylinders are continued in the muscle substance parallel to the long axis of the fibre. These give off very fine fibrils, which form a terminal network within the fibre—named by Gerlach the intravaginal network.

This is not the only form in which the gold salt is strongly reduced by this operation. The characteristic gold deposit is found also in a linear form, and Gerlach considers this effect is produced by changes in a special constituent part of the muscular substance, of which there are thus two kinds.

We are familiar with the latter appearance in preparations of the frog's muscle obtained by the action of ½ per cent. gold solution and subsequent maceration in water strongly acidified by acetic acid. We have no reason to believe that it is more than a deposit in the interstices present in the fibre, and of a nature similar to that found between the bundles and lamellæ of the cornea.

Gerlach, finding that the minute fibrils are sometimes lost in this dark deposit, believes that the muscle substance actually forms a terminal expansion of the nerves; but according to our mode of interpretation it simply indicates that the terminal network interlaces the primary buudles which constitute the fibre. The nuclei of the "terminal plate" (or "end plate") are, we believe, nuclei of flat cells of the fine connective tissue around the nerve, brought into prominence by the deficiency of sarcolemma and muscle substance at this point.

Gerlach states that the application of his method is much more difficult in mammalian muscle on account of the rapid occurrence of the *rigor mortis* and the difficulty of hitting the right time after the death of the animal for placing the fibres in the solution. Successful preparations were obtained by the discoverer in the muscle of the ox and dog. By waiting an hour after death in the former instance and an hour and a half in the latter, the existence of the intra-vaginal network was demonstrated.

For the nerves of tendon Rollett recommends the removal of a small tendon entire, which is then placed in $\frac{1}{10}$ per cent. solution of hydrochloric or nitric acid until it swells into a clear transparent mass. He also recommends a combined action of osmic and nitric acids. The tendon found by him to be specially suitable for the study is that of the musculus sterno-radialis (Cuvier) of the frog. The figures given by this observer of the nerves in tendon, so far as he has followed them, suggest an analogy with those of muscle.[1]

The **terminal bulbs** or **corpuscles** of Krause have been found in the conjunctiva bulbi, the glans penis, the clitoris, and some other parts. Poncet[2] has recently demonstrated them very successfully in the conjunctiva of the human eye by the following method:—He injected $\frac{1}{10}$ per cent. solution of osmic acid under the superior and external part of the con-

[1] Wien. Akad. Sitzungsb., 1876.

[2] "Recherches sur la terminaison des Nerfs dans la Conjonctive." Arch. de Phys., tome ii., 1875.

junctiva where they are best found, producing an artificial chemosis. A few drops of the same solution were then placed between the eyelids, and the parts left undisturbed for 12 hours, after which time the eyeball was enucleated, and small pieces of the conjunctiva removed by scissors. These were extended on cork by pins, and placed 12 hours in purpurine, 12 hours longer in weak alcohol, then 1 hour in strong alcohol, and finally cleared up in oil of cloves, and preserved in soluble Canada balsam. The nerves were found to terminate in—1, a network with large meshes; 2, Krause's corpuscles; 3, in filaments between the epithelial cells.

The memoir of Poncet is otherwise remarkable as affording evidence of the power of osmic acid in favourable circumstances to fix and colour nerve fibrils that have hitherto, we believe, been only seen in gold preparations.

The **Pacinian Corpuscles** are best studied in examples from the mesentery of the cat, in which they are easily seen as small oval bodies about 1 to 2 m.m. in length. They should be examined fresh in serum, and also treated by osmic acid, gold, and the usual staining and hardening agents. The central axis-cylinder may be seen in fresh preparations, and the layers of endothelial cells in the capsule, discovered and described by Hoyer, should be sought in carefully prepared silver preparations. On account of the smallness of the object, the silver solution, distilled water, and glycerine should be applied in succession on the slide, which is then exposed to the action of light in the usual way.

The endothelial layers thus demonstrated afford an excellent example of the nature and arrangement of the flat cells of connective tissue, their easy demonstration in this instance being due to the feeble development of the fibrillary tissue and the loose application of the layers to each other.

For the methods useful in demonstrating the structure of the tactile corpuscles see under *Skin*.

The medullated nerve is preserved intact by the saturated potash solution, as may be readily observed by treating with it a portion of

the sciatic nerve of a frog. To observe more than this requires a degree of success in the experiment which is rarely attained. In exceptionally successful potash preparations there can be seen fine narrow, elongated cells lying parallel to the nerve fibre, and in some of them it would seem as if there were two lines of these, one closely applied to the medulla, and one more externally which probably is part of the cellular sheath. In some of the fibres seen crosswise, the central canal which corresponds to the axis-cylinder is observed to have a border constituted by a substance different from that of the medulla, and resembling the refractive débris which is usually in potash preparations seen external to it.

To see the grey or non-medullated fibres (Remak's fibres), tease out a portion of the sympathetic nerve of one of the higher vertebrata in salt solution. The neurilemma sheath can be separated by treatment with weak acids.

Ganglion cells may be seen by removing the Gasserian ganglion of the frog and examining it fresh. They may be also studied by dissecting out the aorta of a frog, staining it in gold in the usual manner, and teasing it out. The cells stained a deep purple are readily found.

For the ganglion cells of mammalia harden the spinal ganglia in Müller's fluid, cut and tease out.

In favourable preparations the surface of the ganglion cell is seen to be covered by a layer of flat cells, and in gold preparations, deeply stained, the continuation of these cells on the surface of the nerve fibre can sometimes be traced for a short distance.

The **plexuxes of Auerbach and Meissner** can be demonstrated by the gold method, the manipulations being regulated in accordance with those principles which, as we have already explained, must always be kept in view. A small piece of intestine is moderately distended with $\frac{1}{2}$ per cent. gold solution and ligatured. It is then cut out and placed for about an hour in the same solution. The intestine is thus hardened at the same time that the gold permeates its tissue. It is then laid open, washed, and cut into pieces, which are exposed to sunlight in the ordinary acidulated water until they are of a

dark violet colour. The mucous membrane is then easily removed in water, and portions of the muscular layers are carefully torn off by forceps. They are either examined at once in glycerine or after staining in logwood. The ganglion cells are recognised by their large clear nuclei, and the nerve fibrillæ when present by their ordinary characters.

Heart.—The **fibres of Purkinje** can be seen by dissecting out a piece of the endocardium of the sheep, and teasing it out carefully in iodised serum. Another portion should be treated by diluted alcohol. Ranvier considers them as muscle arrested in its development. The anastomoses of the cardiac fibres can be seen in teased preparations. The silver markings which cross the fibres can be seen by tearing off a piece of endocardium, and treating it with silver in the usual way. When treated with 40 per cent. potash solution, the fibres break across at the parts indicated by these lines.

Bloodvessels.—The calibre and arrangement of bloodvessels is best studied in injected preparations, as, for example, in the omentum of a young rabbit injected from the aorta with Berlin blue and gelatine.

Bleed a small animal to death, and inject through the bloodvessels $\frac{1}{4}$ per cent. solution nitrate of silver. Cut out a portion of a transparent vascular tissue, and place it in a drop of glycerine on an object-glass, and examine as soon as the colour darkens. The endothelial markings of the capillaries are usually apparent. Or, simpler, let the silver solution fall drop by drop over the muscles of a frog's thigh which have been just laid bare. Cut off a thin superficial portion of the milky-white surface, place it in glycerine in sunlight, and examine. The silver lines of the bloodvessels are well marked.

Drop gold solution in a similar way, then cut off a portion of muscle, and place it further in gold for 10 to 20 minutes. Expose to light in acidulated water, and when dark in colour examine. The capillary vessels are seen to be composed of a straight homogeneous central portion and an outer, differently

stained and more irregular portion (adventitia). Similar observations may be made on the capillaries of the same part by rapid staining in logwood solution and examination in glycerine. In such preparations nuclei can be seen in this adventitial covering. For the smaller arteries and veins, examine portions of the pia mater hardened in Müller's fluid. Their endothelial lining is seen in silver preparations. The smaller arteries are easily recognised by the peculiar form and transverse position of the nuclei of the involuntary muscle cells. These can be well seen in gold or dye-stained preparations of the omentum or mesentery. In silver preparations the contour of the contractile cell itself is sometimes seen.

In the adventitia of the capillaries in the eye of the frog Mr. Ewart and ourselves, during an investigation of the structure of the retina, observed small narrow elongated cells, similar in form and size to those described and figured by us as lying on the finer bundles of fibrillary tissue in the optic nerve expansion. The eye-ball with the optic nerve cut off short had been placed for 24 hours in $\frac{1}{2}$ per cent. osmic acid solution, and was then dissected and examined in solution of acetate of potash. These cells belong to the category described by us as investing the small or primary bundles in different forms of connective tissue.

To see the elastic membranes, muscular layers, and elastic fibres of the large arteries, harden a part of the thoracic aorta of a large animal (or man), embed and make transverse and vertical sections which can be examined entire or teased out. Or a portion may be pinned out on a piece of cork, rapidly dried at a high temperature, placed between two pieces of cork and cut. The sections are softened in water, and examined.

The structure of the arteries may be also advantageously studied in sections through the tongue of a small rabbit, which has been hardened by chromic acid and stained. The coats are well seen in those which are cut transversely.

The circulation of the blood can be observed in the web of the frog's foot. One or two drops of $\frac{1}{10}$ per cent. solution of curari being injected under the skin, the creature soon becomes motionless. It is then laid on an oblong piece of cork, and the web, moderately extended, is fastened over a small hole cut to allow the passage of the light reflected from the mirror. The cork is fastened on the stage of the microscope, and a piece of wetted cloth laid over the animal. The current in the arteries, capillaries, and veins, and the red and colourless corpuscles are seen without difficulty.

The diapedesis of the corpuscles in inflammation has been chiefly studied in the tongue and mesentery of the frog, the process being observed and described by Waller in 1846, and again by Cohnheim in 1867. It is unnecessary to enter minutely into the details of the operation here, but the principles on which it has been carried out may be shortly explained. The sensibility of the animal is destroyed, and curari injected to paralyse the muscles. A circular hole is punched in the cork oblong, and a similar hole is cut in an ordinary cork of a thickness fully equal to that of the frog's body. This cork is fastened over the hole in the oblong by pins. An opening is then made through the abdominal wall of the frog (a male) about half an inch from the middle line, care being taken to avoid cutting veins, any hæmorrhage which is produced being arrested as rapidly as possible. When the opening is made with a wire heated to a white heat hæmorrhage is effectually prevented. The mesentery is then spread over the opening in the cork. It is more effectually retained in its place when a small raised border, over which the intestine can be secured, has been cut round the edge of the hole.

By a somewhat similar arrangement the circulation in the tongue of the same animal can be observed.

In these transparent tissues it is sometimes possible to trace other structures as well as the bloodvessels. Medullated nerve fibres, for example, may be observed, and at different points the nuclei of cells are seen surrounded by a substance in the interstices of the tissues which refracts light differently from the tissues themselves. It is only when surrounded by this substance that the nuclei are visible, the cell substance being invisible.

Lymphatic vessels.—The larger vessels can be hardened and examined in the usual way. For the finer lymphatic vessels there are two methods; viz., injection and silver impregnation. For the former Ludwig and Schweigger-Seidel recommend the

following method: A rabbit is cut through the middle, the stomach and intestines being removed after a ligature, enclosing the œsophagus and the large vessels, has been passed round the spinal column. The thoracic half of the animal is suspended head downwards by threads passed through the abdominal parietes, and an injection mass (Berlin blue) is poured into the concavity of the diaphragm. The liver is left in its position or drawn slightly upwards by a thread passed round the gall-bladder. After a little time, even if the parts are kept motionless, the lymphatic vessels on the convex surface of the diaphragm are found injected, but this result is more readily attained if a rhythmic movement of the diaphragm is produced. This is done by inserting a tube into the trachea and imitating the movements of respiration.

First, the clefts between the bundles of the centrum tendineum fill with the mass, and afterwards the network of lymphatic vessels which exist on the pleural side.

To see the endothelium of the lymphatic capillaries, lay bare the centrum tendineum of a young rabbit. Brush it gently on both sides with $\frac{1}{2}$ per cent. solution of nitrate of silver to remove the superficial endothelium, cut it out carefully and place it for a minute in $\frac{1}{4}$ or $\frac{1}{2}$ per cent. silver solution. Dip it for an instant in distilled water to remove coagulated matters from the surface, and expose it to light in glycerine until it is of a dark colour. The less it is in contact with water the better, and practically in such manipulations it is often found convenient to neglect the impurities on the surface of the preparation rather than endanger the destruction of delicate elements by washing.

The lymphatic capillaries are distinguished by the peculiar appearance of their endothelium and by their form and course.

There is a source of fallacy to be guarded against in studying the lymphatics by silver impregnation. Wherever a patch of flat cells is seen in a tissue it must not be supposed that there is a lymphatic vessel. The delicate endothelium which covers the bundles may be

demonstrated in part and the appearance of a lymphatic simulated.

This partial demonstration occurs most frequently in the distended interfascicular and interlamellar spaces which are always present in connective tissue, and which are more or less favourable to the action of silver. But it does not follow that these spaces form part of a closed cavity, or that they are part of the lymphatic vascular system. There is, however, a direct passage of solids in a very fine state of division from them to the lymphatic vessels proper.

This is in effect the relation which the straight spaces between the bundles on the abdominal surface of the centrum tendineum, which fill with injection in Ludwig and Schweigger-Seidel's experiment, have to the lymphatics. Although they are lymphatic spaces and are, in a certain sense, in direct communication with the lymphatic vascular system, they are not anatomically part of it. The exact anatomical connection between the lymphatic vessels and the interfascicular spaces (essentially part of the lymphatic system in a physiological sense) has not yet been made out.

A simple method of injecting lymphatic vessels is the puncture method of Hyrtl. A 2 per cent. watery solution of Berlin blue is driven slowly into the tissue by means of a syringe with a very fine canula. In many instances the solution finds its way into the lymphatic vessels. The part is cut out, hardened in alcohol, and prepared for preservation in dammar in the usual way.

A solution of alcannin in turpentine is used by Ludwig for injecting the lymphatics, and is said to possess special advantages.

It may be convenient to notice here the experiments by Arnold,[1] Thoma, Von Wittich, and others, with indigo-carmine. When $\frac{1}{6}$ per cent. solution is injected slowly into the blood under very low pressure in frogs, and the tissues afterwards irrigated with $1\frac{1}{2}$ per cent. salt solution, a dark blue deposit becomes visible. This has been found to take place between the epithelial cells and in the interlamellar and interfascicular spaces, and confirms the view

[1] See Virchow's "Archiv.," vol. lxviii., p. 472, and "Centralblatt," 1875, No. 2.

that plasma from the blood-current accumulates in these positions. For further details the original memoirs can be consulted.

Leo Gerlach found similar results after injecting the solution into the lymph-sacs.

Lymphatic glands.—Harden the fresh gland in equal parts of alcohol and water for 5 or 6 days, the fluid being changed twice or thrice, then for 24 hours in alcohol. Embed and make sections. Or harden in $\frac{1}{3}$ per cent. chromic acid, frequently changed, for a similar time, then for one day in $\frac{1}{2}$ per cent. and another in 1 per cent. solutions.

The sections are shaken gently in a test tube with water, in order to get quit of a number of the lymph cells. The capsule, septa, reticulated cellular substance of the follicles with its lymph cells, the spaces between the follicles and septa, and the supporting fibres which pass from the follicles to the capsule and septa, are seen in such sections.

The bloodvessels are seen in preparations of glands previously injected. The lymph channels can be injected from the afferent lymphatic, which is, however, very difficult to find. It is easier to fill them by "puncture," the point of a fine syringe being inserted under the capsule, and very gradual pressure made. To facilitate their injection, Frey recommends that the animal shall be killed by a blow on the head, the thoracic duct ligatured, and the body lie untouched for 2 to 6 hours. After that time the lymphatic vessels are distended.

Digestive tract.—The Mouth.—The glands of the mucous membrane of the cheek, lip, palate, and pharynx, and the general structural peculiarities of the different forms of papillæ in the tongue, can be seen in alcohol and chromic acid preparations.

The **tonsils** are hardened, cut, and shaken as directed for the lymphatic glands. They should be taken from children. Frey recommends young dogs, swine, and calves.

To see the folds of the mucous membrane and the mucous glands of the œsophagus, Exner recommends specially that of the dog. The œsophagus and trachea of a child or rabbit,

taken out and hardened together, and sections made which include the lumina of both, are instructive.

The submaxillary, lingual, and parotid glands should be hardened in alcohol, and sections variously stained.

The **submaxillary gland** may be selected for careful study. It is hardened in absolute alcohol, and sections stained in carmine and logwood. It should also be macerated in very weak chromic acid, and teased out. Minute portions of the fresh gland may also be teased out in iodised serum.

In the gland elements are to be distinguished the large rounded cells, and more externally smaller granular cells arranged in a crescentic form. Sections of the columnar epithelium of the ducts will be seen.

The membrana propria deserves especial attention. It is to be observed that there is a very fine investing membrane, and that in close connection with the membrane there are flattened nuclei to be observed.

Krause states that the structureless membrane of the acini can be coloured by maceration of the gland in 5 per cent. solution of molybdate of ammonia and subsequent treatment by 40 per cent. solution of tannic acid, or 20 per cent. solution of pyrogallic acid.

The **membrana propria** of this and other glands may be isolated by maceration in weak soda lye which destroys the cells of the gland, but leaves for a time the membrane entire. It also colours yellow with iodine.

Stomach.—Harden a portion of the stomach of a newly-killed animal in absolute alcohol, and another portion in bichromate of potash or chromic acid. Embed and make sections in both directions, stain, and examine in glycerine. The cells of the peptic glands and the surrounding reticulum of connective tissue can be seen. Varied staining with carmine, logwood, and aniline dyes is necessary to examine the different points satisfactorily. Avoid tearing the mucosa away from the muscular coat. In the mucosa sections of the muscularis mucosa

can be easily recognised, and the nuclei are seen in stained preparations.

For the bloodvessels it is necessary to examine the injected stomach. Sections of the frozen tissues and osmic acid preparations further stained in watery solution of aniline blue are also to be recommended for the whole intestinal tract.

The so-called mucous glands of the stomach may be sought for in the pyloric region of the dog, cat, rabbit, or guinea-pig.

Small intestine.—Harden, embed, stain, and cut longitudinally and transversely to the lumen of the bowel. The sections can be distinguished by observing which of the muscular layers is cut transversely: if the inner it is a longitudinal section, if the outer, transverse. Observe the villi and Lieberkühn's crypts.

To see the seam of the epithelium, scrape the inner surface of the intestine of a newly-killed guinea-pig with a sharp knife, or cut small superficial portions with scissors, and examine what is removed in the natural mucus, without further addition. If a cover-glass is used, protect the fragments of villi and isolated cells which are in the fluid by placing a hair or small pieces of thin glass under the edges of the cover-glass, and if the preparation becomes dry add blood serum or aqueous humour.

The relations of the bloodvessels to the villi and Lieberkühn's crypts can be seen only in injected preparations.

The chyle passages may be injected by inserting the point of a fine syringe charged with Berlin blue under the mucous membrane, a small quantity only being injected. They may be further studied by feeding a rat on fatty food and killing it 3 to 4 hours afterwards. The manner of killing must be such that convulsive movements are not produced. A small portion of intestine is then removed and placed in solution of osmic acid until it is quite dark in colour. It may be embedded and cut immediately, or after hardening in alcohol. The small fatty globules are stained black.

The delicate connective tissue of the intestinal mucosa

appears after the action of alcohol and chromic acid as reticulated, and lymph corpuscles are found in its meshes. It has been hence described as adenoid or gland-like. But the tissue is here so delicate that we cannot draw safe conclusions regarding its arrangement and structure from what remains of it after treatment by chromic acid or alcohol; nor do we think that the existence of adenoid tissue as a special type is established. In silver preparations colourless stellate fields can sometimes be observed which will be interpreted according to the ideas the observer may have regarding the significance of this appearance. They tend to show that the tissue is in reality constructed on a plan analogous to that of connective tissue in other parts.

In many animals coloured matter in a reticulated form is found after the action of dyes, especially logwood, extending from the mucosa to spaces between the epithelial cells, and in these inter-epithelial spaces small nuclei can be stained. Lymph cells are also found between the epithelium (Watney). There is thus direct communication between the interepithelial spaces and the mucosa.

Debove has shown the existence of a continuous layer of flat or endothelial cells immediately under the epithelium by the following method: The intestinal mucous membrane is extended, the mucous surface being uppermost, and $\frac{1}{3}$ per cent. solution of nitrate of silver is dropped over it. It is next placed, still extended, for half an hour in distilled water. The brown epithelium is then easily removed in the water. The preparation is again placed in silver solution for some time longer, after which it is quickly washed in water, then transferred, first to alcohol, thence to absolute alcohol, and after the action of turpentine is preserved in dammar.

The method succeeds most easily in the frog, but is also successful in mammalia.[1]

Amongst the columnar epithelial cells of the villi search for individual cells in which the seam is wanting and the anterior

[1] "Archives de Physiologie, 1874."

part of the cell apparently empty. These are the so-called **goblet** or **chalice cells.**

Brunner's glands will be found in sections of the duodenum either hardened by alcohol or chromic acid or cut from frozen tissue.

To see the ganglion cells of Auerbach's plexus cut out a piece of the small intestine of a newly-killed guinea-pig, distend it moderately with air between two ligatures, and place it in $\frac{1}{8}$ per cent. solution of chromic acid. Change the fluid daily for some days, increasing the strength of the solution after the third day to $\frac{1}{4}$ and finally to $\frac{1}{2}$ per cent. Within a week it can be examined. Cut open the intestine, spread it out on a piece of cork with pins, keeping it moist with the solution, and strip off layers from within outwards. The ganglion cells are found between the muscular layers.

(For their demonstration by chloride of gold, see under *Ganglion cells.*)

The isolated follicles which are found scattered through the small intestine, and Peyer's patches at its lower part, require careful hardening in chromic acid.

The crypts of the large intestine can be seen by the same method.

The Pancreas is to be examined by the same methods as the submaxillary gland. The excretory ducts can be injected.

The Liver.—The liver cells can be seen by making a section of the fresh organ with a sharp knife, and teasing out the section, or by macerating a small portion of liver substance in diluted alcohol or Müller's fluid for a few hours. Harden a portion of the liver of any mammalian animal in alcohol (the swine's liver is particularly recommended), and make fine sections and stain them in picrocarminate, logwood, and other dyes. The foramen in the centre of the lobule is a transverse section of a branch of the hepatic vein. Small round apertures between the cells correspond to the capillary bloodvessels. Nuclei stain in the connective tissue, which is between the lobules. It sends in fine projections between the

rows of cells. Treatment by osmic acid and subsequent aniline staining is likely to give good results in examining this delicate form of tissue. The arrangement of the bloodvessels in the lobule is most conveniently seen by injecting from the portal vein. The capillary gall-ducts can be injected, although successful injections are not very common. Asp especially recommends for this purpose a solution of alcannin in turpentine. It is only in injected preparations that they can be studied. The most instructive preparations are from a liver in which both the bloodvessels and the bile-ducts have been injected.

Dr. Rutherford ("Practical Histology," p. 180) states that "Ludwig has recently employed a mass, consisting of asphalt dissolved in chloroform and filtered, for the injection of the bile-ducts. The merit of this fluid is, that chloroform being an extremely mobile fluid, flows readily along the vessels, and that it rapidly evaporates and leaves them filled by a solid black mass."

Spleen.—Place the spleen of a rabbit or guinea-pig for several days in diluted alcohol, then 24 hours in alcohol. Make sections, some of which should be brushed in water or shaken in a test tube. Repeat this process, using instead of alcohol, first $\frac{1}{8}$ per cent. chromic acid solution, and after several days $\frac{1}{4}$ and $\frac{1}{2}$ per cent.

Tease out portions of the spleen of the swine fresh, and after 24 hours' maceration in Müller's fluid. By these processes the general relation of the pulp to the malpighian corpuscles is seen.

The organ should be further injected, hardened and cut.

Dr. Klein[1] has lately described the spleen as being constituted by a honeycomb of cell-membranes, a view which, although different from that hitherto taught, has in its favour that it is in harmony with the latest investigations into the structure of connective tissue. Dr. Klein's method consists in injecting $\frac{1}{2}$ per cent. salt solution by the splenic artery until it passes clear by the vein. Tenth per cent.

[1] "Quart. Journ. Mic. Science, 1875."

solution of osmic acid is then injected, the pressure being maintained for 20 to 30 minutes. The spleen is next placed entire in Müller's fluid and allowed to harden in it for 8 to 10 days. It is then placed in alcohol for a few hours to complete the hardening, and sections are made.

The existence of layers of flattened cells does not exclude the possibility of branched cells also existing in the tissue, but this and other questions raised by Dr. Klein's research must be answered by the investigations which will be naturally suggested by his memoir.

The **thymus** gland should be hardened and cut in the usual way.

Lung.—In small portions of the fresh tissue teased out in salt solution, or after maceration in Müller's fluid, the constituent elements can be recognised. The addition of acetic acid to fresh preparations especially demonstrates the elastic fibres. But useful preparations cannot be obtained without injections. Exner recommends the following method: Take a dead-born child or other mammal (the alveoli consequently not containing air). The air passages are injected with warm cacao butter (the thorax being previously warmed through). As a result of the injection the alveoli acquire the form which they have in life. Whilst the cacao butter is still fluid, the pulmonary arteries are injected from the right ventricle with Berlin blue. The trachea, arteries, and veins must of course be ligatured immediately after injection, or the corresponding canulæ plugged, in order to prevent the injection-mass from flowing backwards. When the tissue is cold the lung is placed in alcohol to harden, and is then cut. The sections are preserved in dammar or Canada balsam, and allowed to lie in the turpentine until all the cacao butter is extracted.

The epithelium of the alveoli is difficult to see on account of its transparency, the nuclei alone staining by the ordinary dyes.

The application of nitrate of silver and the injection of the lymphatics by puncture are both of value in the study of the structure of the lungs, but do not require special description here. The silver may be mixed with warm solution of gelatine

and injected by the trachea. When the gelatine has set, sections are made and exposed to light in glycerine. Sections through the dried tissue are obtained by distending the lung with air and drying rapidly in the sun or near a fire.

Kidneys.—Inject with Berlin blue, harden in alcohol, and make sections through the cortical and medullary substance, some parallel and others transversely to the direction of the pyramids. Observe the glomeruli and the sections of the uriniferous tubes. It is most convenient to select the kidney of a small animal, as sections through the whole organ can be made.

The uriniferous tubes can be isolated by the following method: The kidney is boiled 4 to 8 hours in a mixture of 5 parts by measure of fuming hydrochloric acid and 400 parts absolute alcohol, the flask used being fitted with a cork through which an open glass tube 2½ yards long is passed (to condense the alcohol which passes over). The kidney is then placed in water. The connective tissue is destroyed by the process and the epithelial elements are easily isolable.

Sections of frozen kidney and of the organ hardened in 2 per cent. solution of bichromate of potash should also be made.

The demonstration of the endothelium of Bowman's capsules and of the basement membrane of the uriniferous tubes by nitrate of silver is possible, but often fails. The silver solution may be injected through the bloodvessels and sections of the hardened organ exposed to light. Or freshly-cut surfaces may be treated with the solution in the ordinary way, hardened and exposed to light in alcohol, and then sections made.

The **ureters** and **urinary bladder** by the usual methods.

Testicle.—Prepare and examine by the usual methods. From the testicle of the rat or rabbit cut into halves and placed a few days in weak chromic acid, the seminiferous canals are easily isolable.

The connective tissue of the testicle has been profitably studied by Mihalkowics. He recommends the rabbit's testicle. His methods

consist in injection by interstitial puncture of $\frac{1}{4}$ per cent. osmic acid solution and subsequent examination in solution of acetate of potash; hardening in absolute alcohol and staining sections in hæmatoxyliu; teasing out of fresh preparations, and injections. He found that the bundles of fibrillary tissue between the seminiferous tubes are invested with an endothelium, that endothelial membranes spread themselves over the tissue, and that the vessels and tubes being embraced by the finer bundles are thus actually invested by an endothelial covering.

By teasing out a portion of the fresh testicle in serum, some of the tubes may be isolated, and if they are then treated by nitrate of silver in the usual way their endothelial investment is indicated by the ordinary reticulum.

The **Female generative organs** are examined by means of the ordinary histological methods. In examining the ovary it is advisable to prepare specimens from new-born animals, and at different ages up to full maturity. The elements can be well preserved by hardening the ovary in Müller's fluid. Osmic acid preparations are instructive.

Sections of the ovary of a young kitten are recommended to show the development of the ova from the columnar epithelium on the peritoneal surface. These are inclosed by the growing connective tissue. In sections of the ovary which show the ova in different stages of development, the epithelial cells of the Graafian follicle can be seen, as has been lately pointed out by Foulis and by Kölliker, to be a new formation from the tissues surrounding the ovum.

The exact source of the cells, a problem that involves the general question of the growth of epithelium, has not, as we believe, been in this instance worked out.

The **spinal cord** can be hardened sufficiently for cutting by placing a small portion in a large quantity of $\frac{1}{8}$ per cent. solution of chromic acid. This should be changed occasionally and the strength of the solution after a week gradually increased until it reaches $\frac{1}{2}$ per cent. The consistence is to be occasionally tested, and when the requisite hardness has been

attained, sections are made, or the specimen is kept for future use in diluted alcohol. When the hardening has once reached the proper degree, further action of the chromic acid is injurious and the preparation becomes useless.

The sections are placed in solution of bichromate of potash for several days in order to part with some of the chromic acid and make them fit for staining. It is recommended to begin with the spinal cord of fishes.

A 2 per cent. solution of bichromate of ammonia is also used for hardening the brain and spinal cord.

Gerlach's methods for investigating the fine nerve network of the grey substance are the following :

Small portions of the spinal cord of a child are placed in 1 to 2 per cent. solution of bichromate of ammonia until they are hard (15 to 20 days), the preparation being left in a cool place. Sections are made and placed in a solution of 1 part of chloride of gold and potassium in 10,000 parts of water which has been very slightly acidulated with hydrochloric acid. They are then washed in 1 part of hydrochloric acid in 2000 to 3000 parts water, and placed for 10 minutes in a mixture of 1 part of hydrochloric acid in 1000 parts of ordinary alcohol. Then successively in absolute alcohol, oil of cloves, and Canada balsam. After 3 to 4 hours the nerve network is visible.

A second method : The spinal cord of the ox or calf, whilst still warm, is cut into fresh sections, which are made as thin as possible. These are placed immediately in a solution of 1 part of bichromate of ammonia in 5000 to 10,000 parts of water, in which they are left for 2 to 3 days in a cool place. They are stained by being put in very diluted carmine for 24 hours. The sections are washed with water, teased out, and preserved in glycerine.

The brain is hardened in the same manner as the spinal cord. When it is wished to make sections over a large surface, a broad flat knife must be used, and the section kept moist on the knife by a gentle stream of water.

To harden large portions of brain, put them in absolute alcohol coloured by a few drops of tincture of iodine. When the alcohol is colourless (after a day) add a few drops, and repeat this procedure until the colour of the iodine is retained.

The preparation is then placed in solution of bichromate of potash until it is sufficiently hard to cut.

Sections of the substance of the central-nervous system can be rapidly made transparent by transferring them from spirit to ether, and thence to chloroform, in which they become immediately transparent. They can then be made permanent in dammar or Canada balsam.

Gerlach's gold method, described at page 104, can be applied to the brain in investigating the nerve fibres in the cortical substance. Maceration for a week in glycerine of portions which have been treated by $\frac{1}{10}$ per cent. solution of osmic acid, is recommended for the same purpose. The preparation when examined must be protected from the pressure of the cover-glass.

The nerve-cells and axis-cylinders stain readily in several of the aniline dyes, both in alcoholic and watery solutions.

THE EYE.

In order to examine the relative position of the different structures, make a cut over the equator of a fresh eye with a sharp knife, and let the eye remain in Müller's fluid for 2 to 3 weeks. Cut the hardened bulb into several pieces from before backwards. Embed and make sections. The relative position of the cornea, iris and the layers of the bulb can be examined under a low power. In such sections the iris is applied to the inner surface of the cornea.

The bloodvessels of the eye of a small animal can be injected from the ascending aorta. The lymph spaces can be injected by puncture with soluble Berlin blue from the subchoroidal space. The point of puncture should be equidistant from the corneal border and the equator of the eye.

The cornea.—The anterior epithelium can be isolated by the ordinary methods. It can be examined *in situ* by cutting out the cornea of a frog, staining it by gold or silver, and examining in glycerine with the anterior surface upwards.

In the anterior epithelium of the cornea of young rabbits, treated by gold whilst the eyeball is entire, logwood staining demonstrates minute lozenge-shaped nuclei at various points between the epithelial cells. They may also be observed in the frog's cornea, but not so easily.

The endothelium on the inner surface of Descemet's membrane is delicate and easily destroyed. It may be seen in the frog's cornea, examined fresh in aqueous humour, some hours after the death of the animal, but great care must be taken not to injure it. It can be seen more easily by the following method: Pith a frog, and drop some silver solution over the cornea, or touch it lightly with nitrate of silver. In a few minutes cut its head off, and excise the cornea. Mount it in glycerine with the inner surface upwards. As soon as the light has acted, the large nuclei of the cells and generally some of the cell outlines are visible.

Place the newly-excised cornea of a frog in $\frac{1}{2}$ per cent. silver solution for a few minutes, then dip it in water, and expose it to sunlight in glycerine, the epithelium being gently removed by a small brush or the edge of a knife. The cornea will in this instance be formed of a dark substance intersected by colourless anastomosing figures. The same appearance is seen if the corneal surface is touched by the caustic a few minutes before the death of the animal, and this is the method sometimes recommended for the demonstration of these colourless figures in a dark ground. The application of the silver whilst the animal is alive is unnecessary. It is sufficient if the cornea be taken immediately after the death of the animal.

Sometimes in a silvered cornea, and more frequently if from a young animal, instead of stellate colourless figures, a corresponding appearance of a dark substance formed by the aggregation of minute black granules is observed. The chances of this appearance being obtained are increased if the cornea, after being removed from the silver solution, is placed in $\frac{1}{2}$ per cent. salt solution, instead of water, before being exposed to light in glycerine.

The silver process should be repeated with corneas of different animals and at different ages, and at varying periods after death, and should also be modified as follows: The fresh eyeball is placed entire for a minute in $\frac{1}{2}$ per cent. silver solution, the anterior epithelium is then carefully removed, and the eyeball replaced in the silver solution for a minute or two longer. It is then plunged for a few seconds in $\frac{1}{2}$ per cent. salt solution, after which it is cut out, stained, and examined in glycerine.

The cornea of the frog, mouse, and rat are sufficiently thin to be examined entire. The thicker corneas of larger animals must be transferred from the silver solution to alcohol until they are sufficiently hard to be cut. The entire bulb should in some instances be hardened: in others only the excised cornea.

Corneas prepared by each of these different methods should be placed for several days in 2 per cent. acetic acid. The laminæ can then be detached by fine forceps, and separately examined in glycerine.

By any one of these different methods the ordinary dark colourless figures of the "silvered cornea" can be seen, but to be able to interpret them it is necessary to make use of all the processes.

With the exception of these invariable colourless stellate figures in a dark ground, all the other appearances in the cornea, and the most instructive of them, are obtained only in exceptional instances.

In the corneas which have been silvered whilst the bulb is entire (and sometimes in the others, although much more rarely), the position of the corneal nerves is indicated by long wide, straight, colourless spaces, which become narrower as they approach the centre of the cornea, bifurcate, and are finally lost in the ordinary spaces. One of these straight colourless spaces from the rabbit's cornea is shown in fig. 7, *a*; the silver markings indicating the existence of flat cells are shown at *b*; the continuity with the colourless spaces of the

cornea at *c*; the stained *substantia propria* at *d*; and the ordinary colourless spaces of the silvered cornea at *e*.

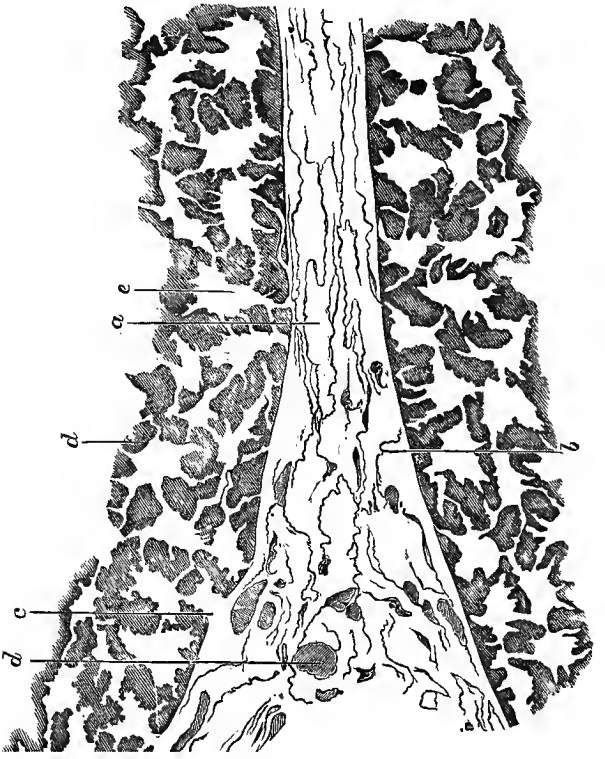

Fig. 7. The sheath of a nerve-trunk and its endothelium in the cornea of a rabbit.

Occasionally, in very successful preparations, in this straight colourless space there is a complete reticulum of the ordinary silver markings, which indicate a connected layer of flat cells, and there is no doubt that they indicate a lymph channel surrounding the nerve. On the border of this lymph channel a

narrow colourless part can sometimes be found, which connects the nerve space and an ordinary stellate colourless space.

In the colourless stellate spaces the narrow silver markings can sometimes, although not often, be seen. Frequently only isolated lines are seen crossing a space. Less frequently lines can be seen joining each other at an angle, and in more fortunate preparations the complete contour of a cell can be observed. In all these instances it is to be noticed that the silver lines have no definite relation to the stellate colourless space. It is crossed by them in every possible variety of manner.

The cornea of young guinea-pigs is well fitted for the production of the silver lines, but for preparations showing their extent and nature, the cornea of the mouse offers unusual advantages. Being very prominent, it can be removed by one

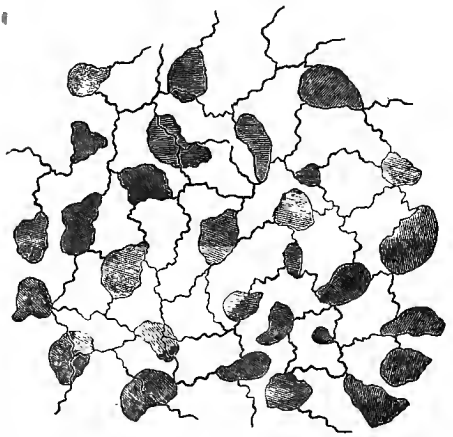

Fig. 8. Complete endothelial layer in the substance of a mouse's cornea, demonstrated by nitrate of silver.

stroke of the scissors. It should be placed for 1 to 3 minutes in $\frac{1}{4}$ or $\frac{1}{2}$ per cent. silver solution, then dipped for a second in salt solution, and immediately placed in sunlight in glycerine.

As soon as it shows the faintest brown tint the epithelium should be removed and the cornea examined. The lines can then be sought for. If not found the process should be repeated on another cornea. There is a stage in the staining which is specially adapted for the study of the silver lines—that, namely, in which the colouring of the ground substance is confined to faint yellowish-brown islands in a colourless field. The lines in the colourless ground form a complete reticulum, which is mostly interrupted by the stained patches. Where the staining is very faint the lines can be traced over and beyond the patch, showing that the interruption of the lines in a stained cornea is due to their being covered and optically obliterated by the dark ground substance.

It is convenient to consider here the interpretation which can be given to the appearances produced by nitrate of silver in the cornea, and how far they can be applied to silver appearances in general.

Black deposit of albuminate of silver is seen in the cornea in three different forms ; as a reticulum of black jagged lines, as large black granules arranged linearly or in anastomosing stellate masses, and as an apparently homogeneous mass coincident with the *substantia propria*. There is to be considered further the uncoloured spaces seen in the substance of the coloured mass.

The meshes of the network are similar in size and arrangement to networks that can in many parts be easily shown to correspond to a layer of cells, and it is therefore fair to assume that in the substance of the cornea the same appearance should receive a similar interpretation. It is occasionally possible to demonstrate a nucleus in the centre of the cell-outline.

The dark granules in linear and stellate form consist of albuminate of silver. In teased out preparations they are found adherent to the lamellæ and bundles, but are easily disturbed by the needle. There is no element with a definite contour, or wall which can be isolated, and within which the granules are contained. They have, in fact, never been isolated as independent bodies, and are simply an aggregation of separate particles.

There is, therefore, no *primâ facie* ground for supposing them to exist as individual elements. Further, by various methods a system of channels in the cornea corresponding to the silver deposit can be

demonstrated by injection. On these grounds the idea that the stellate masses of albuminate of silver are cells ought to be abandoned. If the appearance is still to be characterised as the "cornea corpuscle" it should be borne in mind that the word *corpuscle* has here the same meaning as in the term "bone corpuscle," which is applied to the spaces in bone, but on account of its long association with a mistaken notion regarding the cellular elements of the cornea, the continued use of the term, after the idea it originally expressed may be considered as exploded, is only likely to produce confusion. The terms *cornea corpuscle* and *connective tissue corpuscle* are no longer consistent with precision in histological nomenclature, and must, in course of time, have no other than a historical interest or existence.

The linear arrangement of black granules is susceptible of a similar interpretation. They show that an albuminous substance exists free in the cornea, and is arranged linearly, but as no substance of a corresponding form can be isolated, we cannot assume more from the appearance than that the amorphous matter corresponds to an arrangement of the *substantia propria*. The demonstration of bundles indicates their interstices as the seat of this albuminous substance.

The colourless stellate anastomosing figures show that there is a deficiency in the ground substance of at least a corresponding extent, but they show nothing more. They do not by themselves teach us whether there is absence of structure in these "fields" or whether they correspond to a substance that refuses the silver impregnation. Nor do they teach us whether there is a special wall or membrane, (which must exist if they correspond to a system of closed canals), or whether they are simply spaces left by the apposition of bundles and lamellæ of unequal thickness, or with occasional interrupted continuity. The preparations in which the ground-substance is faintly stained, and in which cellular lines are seen to traverse the spaces in a network, without regard to the contour of the more fully developed spaces, prove convincingly, in our opinion, that the latter are produced where the lamellæ and bundles are not in close contact with each other, and that they do not indicate a system of canals with distinct walls.

The term *cell spaces*, still frequently applied to these colourless fields, is ambiguous, and should be avoided. It is a relict of the old notion, no longer tenable now, that the metallic silver or gold deposit produced between the laminæ of the cornea takes place in cells. Whilst this idea prevailed it was natural to consider the colourless

fields in which this deposit took place as spaces in which the cells were contained, and therefore to designate them cell spaces. But it is not only illogical to continue the use of the term after the meaning which it expressed has been given up, but in the present transition state of histological opinion is likely to retard progress.

In sections of soft fresh tissues more frequently, and occasionally in sections of harder tissues like the cornea or cartilage, which are treated by silver, the surface being stained the usual brownish-black colour, interspersed colourless patches or "fields," analogous to those usually seen within the substance, may be found on the free surface. These superficial fields have projections, or processes, and are frequently jagged in contour. It is certain in this case that they correspond to a substance that refuses the silver impregnation, and the jagged edges show that a portion of this only has been left in the preparation. When this experience is taken in connection with the fact that colourless silver fields are frequently intersected by cellular lines, the inference is allowable that colourless silver fields in general correspond to investing substances, of which layers of flat cells form a part. These substances are usually spoken of as cellular membranes, the membrane being supposed to be formed by the cells. It is certain that in many instances this is not, and perhaps never is, the case, the cells, although sometimes closely agglutinated to the subjacent membrane, not actually forming part of it. Appearances observed by us in the retina of frogs and mammals tend to show that both the cells and the subjacent membrane usually refuse the silver impregnation.

To observe the appearances produced by chloride of gold, cut out the cornea of a frog, mouse, rat, or young guinea-pig, place it 20 to 30 minutes in ½ per cent. gold solution, and then expose it to sunlight in water acidulated with a few drops of acetic acid until it acquires a deep violet colour. After washing it with distilled water place it on an object-glass in glycerine, and with scissors or knife make four incisions from the circumference towards the centre. This is done in order that the cornea may lie flat on the glass. Examine in glycerine after removing the epithelium.

Large flattened slate-coloured nuclei are seen, and frequently around the nuclei a dark violet deposit of a finely granular substance—the result of reduction of the gold-chloride. This

deposit is usually in a stellate form, the figures which it produces anastomosing with each other. But it is also sometimes linear as well as stellate, corresponding to the similar deposit in silver preparations.

Embed corneas which have been treated with gold and make vertical and transverse sections.

In the vertical sections observe that the lamellæ are separated from each other by this gold deposit, and that the lines bounding the lamellæ, although mostly parallel to each other, occasionally rise or fall and join those of the range above or below, showing that the lamellæ are not continuous in their parallel course over the whole cornea.

Tease out thin transverse sections carefully with needles, and observe the characters of the gold precipitate. It will be observed that it is formed in an amorphous substance that has neither definite size nor contour, and does not constitute an individual structure. It is not a cell, therefore, as has been generally taught. The formation of this anastomising gold precipitate—frequently described as the "cornea corpuscle"—affords a convenient method of studying the position of the bundles and lamellæ. There is a nucleus visible in this mass, but it does not belong to it, although the great difficulties connected with the study of the corneal cells have led to their being confounded as parts of a definite structure.

To examine further the nuclei of the cornea the following methods are recommended. Place the eyeball of a rat entire in ½ per cent. gold solution for half an hour, then for several days in weakly acidulated water, excise, and stain it in logwood and examine.

Place the entire eyeball of a small animal in ½ per cent. gold solution for an hour, the epithelium being previously gently removed. Then cut out the cornea, place it on a slide in a large drop of gold solution, put a cover-glass over it, carefully wipe off any of the fluid that may escape beyond the latter, and run Brunswick black round the edge. Examine at intervals during the first 24 hours. The nuclei, although not stained, are visible, and in a successful preparation of this kind a much greater number of nuclei are seen than when the ordinary staining processes are used. Especially

along the nerves numbers of narrow elongated nuclei are observed. Stain the cornea in purpurine solution and examine the nuclei. The rat's cornea is especially adapted for this staining, and we have obtained preparations from this animal, in which an extraordinary number of nuclei on the nerves were coloured.

There are other methods by which essential points regarding the structure of the cornea are demonstrable, but success by them is so rare that they are hardly to be undertaken by beginners. Amongst these are sealing sections through the fresh cornea in $\frac{1}{2}$ and in 10 per cent. salt solutions, sealing obliquely-cut fresh sections in aqueous humour and examining frequently during the first three days afterwards, in order to detect cellular elements of the cornea (rounded, polygonal, and elongated narrow cells).

Inject $\frac{1}{4}$ per cent. gold solution from the aorta or carotid of a young rabbit, until the eyeball is very tense, and after half-an-hour cut out the eyeball, place it for a day in acidulated water, and then excise the cornea and examine it in glycerine. In such preparations fine fibres are to be looked for in the cornea substance and minute oval nuclei, which are very different from the nuclei usually seen in cornea preparations. Königstein ("Vienna Academy's Transactions, 1875") recommends the following method : A cornea which has been stained by gold is placed in a mixture of equal parts of the hydrochloric acid of commerce and distilled water, a few drops of glycerine being added. After some hours, or as soon as the cornea-substance falls to pieces when dragged through the fluid by forceps, the lamellæ and portions of them are examined in the fluid. Königstein found appearances which he describes as anastomising branched corpuscles. What we found by a short trial of this method was that delicate minute cylindrical fibres, having the optical characters of elastic fibres, are seen thickly interwoven with the fibrillary corneal tissue. The stellate amorphous gold deposit was unchanged.

The true stellate cells of the cornea (bodies with a small amount of protoplasm and uniform delicate cylindrical processes of a calibre not greater than that of the elastic fibres found in serous membranes, and much resembling them in appearance), are hardly, as far as we know, to be successfully demonstrated except after the spaces between the bundles and lamellæ have been distended by inflammation. They have not been described except by ourselves. Figures 9 and 10, from preparations of the inflamed rabbit's cornea, show the effects of gold and osmic acid solutions in demonstrating these cells when the action of the fluids has been facilitated by distension of the spaces between the lamellæ.

The stellate cells of the cornea can further be sometimes recognised in thin transverse sections through the fresh ox cornea, mounted in aqueous humour, and examined with a good immersion lens a few hours afterwards. But the appearance then seen is only

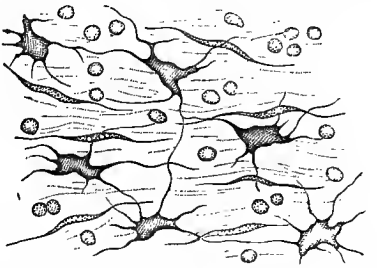

Fig. 9. Stellate cells in an inflamed rabbit's cornea, demonstrated by osmic acid and aniline red. The small round bodies are colourless blood corpuscles.

valuable in so far as it can be controlled by gold and osmic acid preparations.

We believe that there are further minute spindle cells in the cornea, and that their nuclei constitute the dagger-shaped figures seen in preparations of the inflamed cornea. The nuclei of these

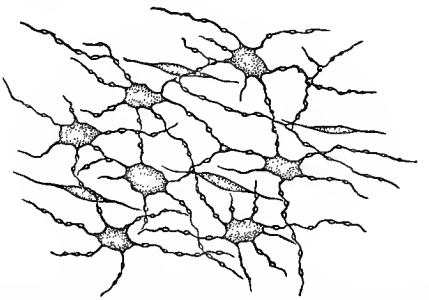

Fig. 10. Stellate cells in an inflamed rabbit's cornea, demonstrated by gold. (The processes in the preparation are dark throughout.)

cells are seen in gold-injected preparations, and occasionally fine processes can be followed on each side of the nucleus. This appearance is more decided in osmic acid preparations of the inflamed cornea, when the sections are very thin and subsequently stained in

red aniline. The obvious objection that may be raised that we have to do with the nucleus and body of a flat cell seen in profile, can only be effectually answered when an easy method is discovered of isolating the figures. At the same time, we have good grounds for describing them as spindle or fusiform cells.

When the cornea substance is rapidly disintegrated by strong acids or alkalies after a metallic deposit has been produced by gold in the interlamellar spaces, the membranous substance to which the deposit adheres resists the solvent action longer than the bundles of tissue, and is accordingly isolated.

These membranous shreds were at one time mistaken for the walls of canals, and have been also considered as cellular elements. The appearance is analogous to the so-called "bone corpuscle" isolated by warm soda lye from bone. Tubular membranous substances, isolated from the retina, and described as radial fibres (but which we shall afterwards see are not the true fibres), fall within the same category. The fact that nuclei may adhere to them, makes the appearance the more illusory. They are not cellular structures, and the form in which they are observed depends on the fact that the membrane which faces the spaces is isolated, while its continuations between the bundles and more compact portions of the lamellæ are severed.

The large delicate flat cells demonstrated in the healthy cornea by silver, and the stellate and spindle cells we have just described, do not exhaust the category of cornea cells. If the cornea of the ox is treated by saturated potash solution, and the operation is successful, narrow elongated cells are isolated which had never been seen until we observed them by this method. The longest of them are of great length, and very narrow, and numbers are found which have an uniform length and breadth. Two, in rare cases three, of these long narrow cells are sometimes found isolated and sutured to each other, end to end. In isolated cells a fine serrated edge can be seen at the extremity, by means of which the suture is effected. The nucleus is oval, is situated at one end of the cell, and occupies nearly its whole breadth. Large fields of

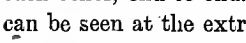

Fig. 11. Elongated narrow cell from an ox cornea, demonstrated by saturated solution of potash.

these cells are seen lying parallel to each other, and in contact laterally, and it is often to be observed that at one end they are sutured to small rounded cells.

In addition to these narrow elongated cells, others of the size and form of ordinary endothelium separated or joined in groups are preserved and isolated by the same procedure.

We are accustomed to apply this method to the cornea in the following manner: Fresh eyes of the sheep or ox give more frequent success than the cornea of smaller animals. If the anterior epithelium is removed before operating, this should be done whilst the eyeball is entire, and 12 hours after death it is easily effected by scraping the surface of the cornea with a scalpel, beginning at one border and carrying off the whole of the epithelium regularly towards the other. The cornea should be injured in this process as little as possible. In a successful operation, after the epithelium is removed, masses of the narrow elongated cells and others of more ordinary form are still found. If the epithelium has been left intact, it is seen in the preparation, always provided the experiment has been successful, and it is invariably found unaltered, the cells having the same size and shape which distinguish them when isolated by the ordinary methods.

The excised cornea, entire or cut into several pieces, is laid in aqueous or vitreous humour saved for the purpose until the solution is ready. A small vessel thoroughly dry is prepared, and a thermometer is suspended over it, in such a manner that the bulb will be in the upper stratum of the solution. The caustic potash (not less than 15 grammes) is ground to powder in a mortar, and an equal weight of distilled water (as many cubic centimeters as there are grammes of potash) is poured over it, and rapid solution favoured by stirring. As soon as the potash is dissolved, the solution is poured into the vessel, and the thermometer watched. If the temperature runs up rapidly to 135° or 140° Fahrenheit, it is of good augury; if not over 120°, it augurs badly; if not over 110°, it will hardly succeed—unless indeed the potash used has been purified in

the manufacture by preparation with alcohol: in which case we do not lay so much weight on the amount of heat generated by the solution as a test of the probable success of the experiment.

As the thermometer falls, the cornea is laid on a cover-glass to remove the excess of aqueous or vitreous humour, and when the temperature has decreased to 107° Fahrenheit, is placed in the fluid. In a few minutes it is ready for examination, a small portion being removed on the point of a needle, and broken up in a drop of the solution.

Whether the experiment is to be a successful one, can after a little experience be inferred from the appearance of the cornea after it has been acted on by the potash. If it flattens out on the surface of the fluid as a pulpy gelatinous-looking mass, it is either for the most part or entirely a failure. In a perfectly successful experiment, the cornea as it contracts increases in thickness, has a white granular appearance, and the little masses detached by the needle have no adhesion to each other.

The success of the experiment can in no case be calculated on beforehand, and depends on conditions, some of which are unknown to us. An important one is chemical purity of the caustic potash, and another is its absolute dryness. We have never succeeded with potash bought from the retail shops. Our first and very successful series of experiments were made with *potassa fusa* in stick, obtained from a well-known firm of manufacturing chemists; but latterly the potash bought from the same firm has been invariably a failure, a fact anticipated by us at the first trial on account of the comparatively small degree of heat generated in the process of solution. This, it has been explained to us, is due to the source of potash for wholesale houses having changed from Canada to Germany, and to the new supply being chemically less pure than the former one. We have therefore been obliged to use the more expensive potash which is purified by alcohol, and by its use we are able to get a fair proportion of successful preparations.

Other conditions are evidently connected with the cornea itself. It has often happened to us, that of a number of corneas removed from animals killed at the same time, kept in the same vessel until used, and treated by solutions of the same sample of potash, one succeeded, and all the others failed. Of a number of frogs' eyes, put entire into the solution in order to examine another tissue, a successful demonstration of the corneal cells was only found in one. (We had learned at an early stage of our experiments that success is seldom obtained from the frog's cornea.)

The rapidity with which the solution comes into contact with the substance of the cornea may have some influence in the matter, as although success may be obtained after the anterior epithelium has been completely removed, we have reason to believe (we have not been able yet to establish the fact with certainty) that the chances of success are increased if the epithelium is left in its place. As in this case the epithelium can be afterwards isolated entire in masses, it is evident that it must when *in situ* have an influence on the rapidity with which the solution penetrates the cornea in the first instance.

In connection with the relation which these cells have to the corneal substance, it is to be noted that they are often found, even when isolated, joined end to end, and that many of them have the same breadth as the primary bundles of the corneal substance. These facts suggest that the cells may be closely applied to the surface of the bundles, a theory that receives confirmation from an observation we have lately made. Whilst examining a portion of a cornea which had been for a very short time in solution of potash purified by alcohol, the drop of fluid used being a large one, we observed the corneal substance undergoing a process of solution. The portion under examination, when first seen, consisted of a large mass of straight, parallel, extremely fine fibrillæ. As these gradually disappeared, and as the part became more transparent, an arrangement of bundles was indistinctly seen for an

instant, and as this vanished nothing was left but a large mass of narrow elongated cells which occupied the position of the bundles.

Layers of small rounded or hexagonal cells, similar to some of the patches indicated by potash, can be sometimes observed in the cornea after inflammation which has lasted for a very short time. Fig. 12 shows part of such a layer from the cornea of a rabbit which had been 4 hours inflamed, and, after excision, treated by gold, and exposed to light in acidulated water. By altering the focus, several similar layers

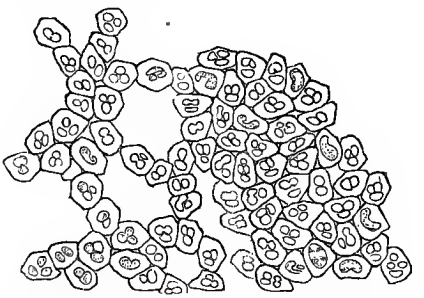

Fig. 12. Layer of flat cells in an inflamed rabbit's cornea, demonstrated by gold. The demonstration is complete on the right of the figure, and incomplete on the left.

were seen above each other. The division of the nuclei is due to the inflammation. We have observed similar cells in the cornea of the mouse after a very slight degree of inflammation.

The same cells arranged in a reticulated form had been observed in the inflamed cornea for some years back, but were considered by the observers who described them as being cells newly formed under the stimulus of inflammation. We showed in 1875 that the reticulum is due to an imperfect demonstration, that the cells really exist as layers, and that they are pre-existing elements of the healthy cornea.

We have also seen in gold preparations the narrow elongated cells in the frog's cornea after slight inflammation, but

are disposed to believe that success was in this instance very exceptional.

To demonstrate the fibrillæ and the bundles which they form, macerate sections of the fresh ox cornea in 10 per cent. salt solution. The fibrillary substance is arranged in thin layers which cross each other at an angle. To demonstrate this fact Schweigger-Seidel has recommended interstitial injections of 1 per cent. tannic acid or diluted alcohol, sections being made through the part injected. The same arrangement can be seen in the spindle-shaped nuclei which are so easily demonstrated by gold in the inflamed cornea.

The bundles may sometimes be isolated by carefully teasing out portions of a frog's cornea fresh in indifferent fluids.

If parts of an ox cornea are placed for an hour in gold solution and macerated over a period of many weeks in water acidulated with acetic acid, they may be first separated into lamellæ, and these may after a time be separated by needles into bundles of about the breadth of the ordinary tendon bundle. These bundles are however really compound, and can be further disintegrated into finer or primary bundles of a breadth slightly less than that of a human red blood corpuscle. They may be observed with more ease by staining them in picric acid.

Descemet's membrane is seen without difficulty in vertical sections through the cornea. Interesting points in its structure, observed after maceration in 10 per cent. salt solution, have been described and figured by Schweigger-Seidel in the "Leipzig Arbeiten" for 1870.

Sclerotic, usual methods.

The **choroid** and **iris** should be first studied in albino rabbits and new-born infants, and by the usual methods. The blood-vessels should be studied in injected preparations.

Between the choroid and sclerotic there is a lymph space (perichoroidal space of Schwalbe), and both on the sclerotic and choroid surfaces an endothelium can be demonstrated by nitrate of silver. Albino rabbits and swine's eyes are suitable for the purpose. Cut the eye through its antero-posterior axis with large, sharp, strong scissors. Let the vitreous humour fall out,

remove the lens and cornea and place the rest of the hemisphere in the silver solution. With the handle of a scalpel open out gently the choroid from the sclerotic so as to let the solution penetrate between them. Then remove carefully parts of both surfaces and expose them to sunlight in glycerine. Examine as soon as the colour darkens.

Similar cellular membranes can be separated in the substance of the choroid, and Key and Retzius state that it is possible to inject different layers between these membranes. Fine trabeculæ cross the perichoroidal space from the choroid to the sclerotic, with the tissue of which they become blended. The trabeculæ are covered with a layer of endothelial cells.

The **retina**.—The methods suitable for this structure may be conveniently tested in the retina of the frog.

If a portion of a frog's retina, carefully removed while perfectly fresh, is examined in aqueous humour or glycerine, the outer segments of the rods can be readily recognised, and on the edge of the portion examined other parts of the retina can be more or less distinctly seen. For further study it is advisable to have recourse to reagents.

Sections of the hardened retina cut by a knife in the usual manner are not so suitable for examination as those which are obtained by carefully teasing out portions by needles. Some of the fragments obtained by the latter method will be found to extend as thin sections through the whole thickness. Others consist of one or more isolated layers, and they can be recognised and studied.

Retina preparations should be examined in a large drop of glycerine in order to protect them from the pressure of the cover-glass. This is useful for another reason. By touching the edge of the glass gently with a needle the element under examination can be made to roll over, and be thus seen in various positions. Preparations that are to be mounted permanently must be protected by the insertion of small pieces of thin glass or the usual paper diaphragm between the cover and object-glasses.

To harden the retina in Müller's fluid or weak chromic acid place the eye in a large quantity of the solution, which should be changed several times during the following two or three weeks.

The retina, hardened in chromic acid, may be placed on an object-glass and successive sections made by cutting it with a sharp scalpel.

It is difficult to give precise instructions for the strength of the acid. It should be used weak at first and may be afterwards used stronger, especially when the eyeball is a large one. The quantity used should be large in proportion to the size of the eye. When the right degree of hardening has been attained chromic acid preparations are highly instructive, but many of them are comparative failures.

The hardened retina may also be soaked in gum-water for 24 hours, placed 24 hours in alcohol, embedded, cut, the sections placed in water for 24 hours to free them from gum, stained and examined in glycerine. The relative arrangement of the different layers and the stronger parts of the radial fibres can be seen in such sections.

Alcohol in various degrees of dilution is also a valuable reagent.

The study of the retina cannot be begun better than by the use of osmic acid. It is to be employed in two different ways.[1]

Excise the cornea of a fresh eyeball of a frog and remove the lens gently. Let the vitreous humour fall off the retina by tilting the bulb, which is then placed in a very small porcelain vessel containing $\frac{1}{2}$ per cent. solution of osmic acid. A capsule of a calibre very slightly in excess of the bulb spares the acid, 4 or 5 drops being then sufficient. Cover the capsule until the retina assumes a black colour. It can then be removed and broken up in glycerine and examined.

By this mode of preparation the structures from the pigment

[1] The description of the retina given in the text is based on experience acquired in the investigations which formed the foundation of a joint memoir by Mr. Ewart, M.B., and ourselves, published in the "Journal of Anatomy and Physiology" for 1876.

epithelium to the intergranular layer can be advantageously examined, and it is advisable to stain some of the preparations by drawing logwood through them with filter-paper. Note that between the outer and inner segments of the rod a granular substance intervenes, and that in the outer end of the inner segment there is a more resistant portion which stains like the outer segment. This is the lens-shaped body of Max Schultze. The intervening granular substance is cellular, as is shown by another method. The lens-shaped body, as was recognised long ago by H. Müller, is simply a more resistant portion of the inner segment. A thin line at the inner end of the inner segment is part of the external limiting membrane, or a fraction of one of the fine fibres which run in it. Two nuclei are to be distinguished here. One is found lying across the end of the inner segment when the latter is detached, and is an element specially connected with the limiting membrane. It is removed along with the rod which is planted on it. The other belongs to the external granular layer. Their relative position can be easily recognised when they happen to be both preserved, which however does not occur frequently.

Between the inner segments of the rods the thin cones are seen.

In the external granular layer immediately inwards from each rod there is a conical structure, the apex inwards, which is often detached in a piece with the rod and intervening portion of the external limiting membrane. Hannover describes it as the *calotte* or night-cap, a name suggested by its form. Mr. Ewart and ourselves have described it as the *outer rod-pedicle*. Its apex is continuous with that of another conical structure, whose base is on the intergranular layer, and which we have designated the *inner rod-pedicle*. This inner pedicle is much less resistant than the outer and is preserved only in exceptionally good preparations. When it is preserved the outer and inner pedicles are seen to form continuous parts of one structure. But the outer pedicle contains a kernel of resistant substance (analogous to the lens-shaped body of the inner segment of the

rod), and is therefore frequently found when no trace of the inner pedicle has been left. In the elliptical spaces left by the diminution in size of the rod-pedicles in the middle of the intergranular layer there is a corresponding element, thickest in the middle and tapering towards the outer and inner ends. Outwards it is in a line with the cones, and we have designated it the *cone-pedicle.*

In these preparations more or less of the radial fibres and the structures between the internal limiting membrane and the internal granular layer are also seen. The internal granular layer especially can be sometimes well studied in such preparations. Round nuclei with a trace of surrounding cell-substance are found inclosed by meshes of minute fibres which can be traced to a radial fibre as their stem. The inner part of the retina, however, requires a special modification of the osmic acid treatment; as follows:

The optic nerve is cut away with sharp curved scissors from the freshly-excised eye close to the sclerotic, and the bulb is placed entire for 24 hours in the osmic acid solution. The object of this manœuvre is that the solution shall find its way to the retina by slowly penetrating inwards along the optic nerve. In doing so it fixes delicate elements that have not been seen by any other method.

The eyeball is then opened in a drop of concentrated solution of acetate of potash, the lens carefully removed, and the bulb cut into several pieces. Fragments of the retina are removed and gently teased out into two or more layers, which are examined in a fresh drop of the acetate of potash solution.

Conspicuous in such preparations is a layer of fine fibrillary tissue on the inner surface of the retina. It corresponds to the optic nerve layer, although by this mode of preparation the nerve elements proper were not seen by us. In some of these preparations narrow elongated cells are seen lying on fine bundles of this fibrillary tissue of the optic nerve expansion. Nucleus and cell contour are distinct and even, and there is no trace of a cell process. The molecular layer is seen as a finely-granular

consistent mass. A layer of rounded cells with large nuclei completely investing its outer surface are often seen in such preparations, and in the angular points between the cells broken ends of radial fibres can be observed. The fibres are slightly swollen at the point where they break and send off horizontally very fine fibres, which encircle the cells and anastomose with each other.

A similar layer of cells which invest the inner surface of the molecular layer may be sometimes seen in part by this method, but can be seen better by other methods.

A number of eyes treated by both osmic acid methods should be placed in glycerine, the retina being allowed to remain undisturbed *in situ* for a period of from 1 to 2 months. Portions should then be removed and examined in glycerine, logwood staining being also used. The effect of the maceration in glycerine is to remove the less resistant parts of the retinal substances, and to render it possible to study the disposition of the radial fibres. The nuclei in these fibres stain in logwood.

The most resistant part or stem fibre extends from the inner surface of the molecular layer to the intergranular layer, and in the internal granular layer contains an elliptical nucleus in its substance. Inwards from the molecular layer it divides into branches, which become very fine. These finally ramify in the internal limiting membrane and anastomose there freely with those from the other fibres. Fine branches are given off abundantly in the internal granular layer and in the intergranular layer. At this point the main fibre divides into finer branches, which pass outwards through the external granular layer, many of them again dividing and reuniting in its substance, so as to form frameworks which surround and support the rod and cone pedicles. Fine branches are given off in the external limiting membrane, and here the radial fibres were previously supposed to terminate. In macerated preparations we have however traced the fibres still further ontwards. After passing through the external limiting membrane they pass along the surface of the cones and inner seg-

ments of the rods and penetrate to the outer segments of the rods.

We have in well-macerated preparations found a reticulum of fine fibres in the fibrillary substance of the optic nerve layer and in the molecular layer. We have also in teased-out preparations isolated fine fibres extending along the outer segments of the rods to the pigment epithelium, with pigment molecules adherent to them. These fibres were in all instances identical in appearance with the finer branches of the radial fibres seen in the layer of rods and cones, and probably belong to that system, although we were not able to trace the connection.

In the same macerated preparations large, flat, oval nuclei of the intergranular layer are to be looked for, and also nuclei of the cells of the external limiting membrane. Both in these and in ordinary osmic acid preparations isolated, elongated, narrow cells are to be looked for in the external granular layer and between the external limiting membrane and the line of junction of the outer and inner segments of the rods. Success is in this point exceptional.

In retinas treated by osmic acid, macerated in glycerine, stained in logwood, and teased out, some of the sections show the structure of the molecular layer from within outwards in layers or strata, which stain dark blue-violet and are separated by colourless spaces of equal and uniform breadth.

In the hen's retina, mounted fresh in glycerine, we have after 24 hours observed this layer to be composed of fine uniform cylindrical bands or bundles parallel to the surface of the retina. As the existence of strata has been observed in the hen's retina, it follows that the substance of the molecular layer consists of parallel strata of fine cylindrical elements, and is thus in intimate relationship with typical forms of connective tissue.

Amongst the rods in osmic acid preparations, are to be observed exceptional rods described by Schwalbe, in which a long, thin, narrow inner segment ends in a short outer seg-

ment. When the outer segments fall off in a mass from the inner segments, they are left attached to the retina. The line of division between the outer and inner segments does not cross them. They must therefore pierce the membrane which we have described as separating the outer and inner segments of the rods, in the points left between small round cells found by us in this position, probably accompanying the radial fibres.

The effects of diluted alcohol in fitting the retina for examination may be considered along with purpurine. Purpurine solution contains a fourth part of its volume of alcohol, and alum to nearly half per cent., and it is highly probable that many of the results published in the memoir previously referred to as having been obtained by purpurine, could also be obtained by diluted alcohol with ½ per cent. alum.

Place the eyeball entire in purpurine solution for 24 hours. Then excise the cornea and remove the lens. Place the bulb, with the retina still in its place, in the solution for 24 hours longer. The retina is then stained a rose colour. Several eyes treated in this way should be placed in glycerine for 1 to 2 months, with the retina intact. Parts of the retina should also be examined at once, carefully teased out, and further stained in logwood.

Retina preparations treated by purpurine (and diluted alcohol, but our experience chiefly refers to purpurine) are very valuable for several important points. The radial fibres of the retina are described and figured by authors in two forms. In one there is a wide funnel-shaped structure, with a trumpet end towards the inner surface of the retina: in the other there is a straight, clean-contoured, delicate fibre. Nuclei are described on the former, and in the latter. Purpurine preparations show that not only both forms exist, but that the one is contained within the other. The funnel-shaped structure is membranous, and the true radial fibres are contained within it. When the retina is examined in iodised serum, the former is seen: when after the action of the ordinary harden-

ing agents, the latter. In purpurine preparations, the true fibres can be distinguished in the folds of the other.

In macerated purpurine preparations, nuclei can be seen in the molecular layer, and nuclei can be seen adherent to radial fibres and membranous débris external to the external limiting membrane.

The outer rod-pedicle and the cone-pedicle are found isolated in such preparations, the former in its "night-cap" relation to the rod. They should be further stained in logwood. They are found in two conditions. In one they are invested by a substance which stains freely, and in which a nucleus may sometimes be detected: in the other they are freed from this substance and have no nucleus, and are little susceptible of staining.

Retina preparations may be divided into two classes, according to the reagents employed. If these are of such a nature that the radial fibres are left intact, whilst the cells and ground substance of the different layers are loosened from their connections or partially disintegrated, we have preparations consisting of a stem radial fibre and the stronger of the dependent fibrils which spring from it. The latter bring away with them in their meshes rods and cones and their pedicles, fragments of the membrane of the intergranular layer and cells of the internal granular layer. If one fibre brings away with it in a teased-out preparation all these parts in continuity from without inwards, we have the appearance which has been construed as forming the unbroken connection between supposed nerve elements in the internal granular layer and the "nerve epithelium." This idea is incompatible with a right understanding of the nature of the different layers.

In retinas which have been for several days to several weeks in a mixture of 1 part methylated alcohol and 3 parts distilled water, and containing $\frac{1}{2}$ per cent. alum in solution, teased out in glycerine and stained in logwood, a radial fibre characterised by the lozenge-shaped nucleus in its substance may be seen to split and encircle a round cell with a large

nucleus, and join again at the opposite pole to form a single fibre. With the exception of the characteristic lozenge-shaped nucleus which had been overlooked, this appearance corresponds with some of the bi-polar ganglion cells which have been figured as being found in the internal granular layer. Most of these are simply one of the flat round cells of this layer isolated in a mesh of a radial fibre.

In the same preparations and in osmic acid preparations, macerated and stained by logwood, small spindle cells, consisting of an elliptical nucleus with scarcely any cell substance and fine single terminal processes, may be isolated. Those of the so-called bi-polar ganglion cells which are not formed by the radial fibre and a cell, are to be referred to these.

Nerve elements have not yet been traced to the internal granular layer, and the assumed continuity between the ganglion cells and the rods and cones has no anatomical basis.

The second class of retina preparations is well illustrated by the action of formic acid and saturated solution of caustic potash. The retina is to be treated with gold and formic acid, by a method modified from that recommended by Löwit to demonstrate nerve-fibrillæ. The cornea and lens being removed, the bulb is placed in $\frac{1}{2}$ per cent. gold solution for 5 minutes, then for a few minutes in formic acid of specific gravity 1020. The retina is then easily detached entire. This done, it is placed in $1\frac{1}{2}$ per cent. gold solution for 10 to 20 minutes, and then in a dark place for 24 hours in 8 to 10 cub. centim. of equal parts of the formic acid and water.

The process is repeated with the bulb entire, the optic nerve being cut close to the sclerotic, and the duration of the action of both the gold solutions being prolonged a considerable time in order to allow the retina to be permeated.

The retinas thus treated by gold and formic acid are readily split into layers by needles. On the inner surface of the molecular layer a distinct layer of investing cells are seen, and on and amongst them the ganglion cells.

From the intergranular layer a membrane may be isolated with nuclei on it, and in the external granular layer rows of isolated cells may be seen, the intercellular substances having been removed by the solvent action of the formic acid.

In such preparations we have observed the molecular layer as a fibrillary substance arranged in fine bundles, on which minute cellular elements were seen; but this effect is very rarely produced.

In a retina which was allowed to remain in the diluted formic acid for eight days, the intergranular layer and external limiting membrane were seen as true homogeneous membranous substances, having been swollen by the long maceration in the acid.

To obtain the effects of saturated potash solution the eyeball is placed in it at the usual temperature (106° to 108° Fahr.). (See further under cornea, p. 117.) After a few minutes the eyeball is opened in a drop of the solution by needles. The retina is readily recognised, and is examined in a fresh drop. If successful the pigment epithelium and the rods usually come away in connected masses as clear and clean as objects chiselled from marble. Between the outer and inner segments of the rods a third block of substance is seen intercalated, which we have attributed to the lens-shaped body. When the outer segments separate in a mass from the inner segments a round nucleated cell is seen on the inner ends of each of the former. Two kinds of cells are seen in the external granular layer, a rounded cell with a large nucleus and a small amount of cell substance, and a small narrow elongated cell having its nucleus at a broader end and tapering to a point at the other. The potash solution sometimes preserves intercellular substance extending outwards from the intergranular layer, and apparently belonging to the inner rod pedicle. An exactly similar appearance was not seen by other methods: none of them preserving so much of the delicate intercellular substances.

When the rod is isolated in such preparations the nucleus of the cell of the external limiting membrane generally adheres

to its inner end. Narrow elongated investing cells can sometimes be seen adherent to the inner segment of the rod, extending from its inner to its outer end, and investing it on both its flattened surfaces.

The internal granular layer is seen to be formed of separate layers of rounded cells, which are characterised by a round nucleus and a scant ring of cell substance. The molecular layer is seen to be invested on the inner and outer surfaces by distinct and complete layers of cells. The ganglion cells are also observed on its inner surface.

The accurate limitation of the pigment at a line equivalent to the outer ends of the inner segments is well seen in potash preparations.

These are not all the points which can be demonstrated by potash, but they will suffice to act as landmarks which will enable observers to localise other appearances which they may see.

The internal limiting membrane is usually difficult of detection by any method, but should always be looked for. It is covered by a layer of cells both on its inner and outer aspect.

These can be seen in the retina of the sheep by the following method: Cut the fresh eyeball through its axis. Let the vitreous humour fall off one of the halves. Mark off a small piece of the retina with the point of a sharp knife, and remove it with care to an object-glass, on which a large drop of carbolised serum has been laid, the internal surface being uppermost. Protect with portions of broken cover-glass or other convenient substance, cover with a very thin glass, and seal with Brunswick black. Examine the following day with a good lens, and if the preparation has been successful a double layer of hexagonal cells, separated by a transparent formless substance, can be seen.

Osmic acid and gold preparations of the frog's retina, in which the solutions have penetrated by the optic nerve, sometimes show these cells. They are hexagonal, and the nuclei are in this case distinct. They were first described by Han-

nover more than thirty years ago. This anatomist observed them in mammalian retinas, examined fresh and warm, without a cover-glass. Their existence was placed in doubt by subsequent observers, and Hannover's description, indeed, almost completely lost sight of.

In the frog's retina they can also be demonstrated by silver. The lens being carefully removed from the fresh eye, the eyeball is placed for a few seconds in $\frac{1}{4}$ to $\frac{1}{2}$ per cent. silver solution. The retina is then carefully removed, and placed immediately on an object-glass in glycerine, and exposed to sunlight. After a few minutes the colour begins to change, and it should be at once examined. The outlines of the cells can often be observed as they appear. Such preparations are difficult to keep. None of ours lasted longer than a few hours.

The same cells can sometimes be observed when the silver solution has been injected from the aorta. In this case the nuclei of the cells may be visible, and their relation to blood-vessels and the optic nerve layer is very distinct.

In the mammalian retina, treated by silver, the cells of the internal limiting membrane usually disappear, and the inner surface is bounded by a colourless substance, intersected by a delicate reticulum of fine lines. These owe their existence to a deposit of albuminate of silver in minute channels on the surface of the membrane, in which the anastomosing terminal branches of the radial fibres appear to ramify.

The ganglion cells can be seen by most modes of preparation, and in all carefully hardened retinas. Like the nerve fibres, they are best studied fresh in vitreous humour. The sheep's retina is suitable for this purpose.

When the external surface of the sheep's retina is placed upwards we have found it possible to observe the cells of the external limiting membrane, the rods being guides for its localisation, but for studies of this kind much patient observation and very careful manipulation are necessary.

The fresh retina of the frog and osmic acid preparations of the ox retina are useful for the study of the pigment epithe-

lium. The minute particles of pigment adhere to the inner surface of the cells, but we do not believe that they penetrate the cell, which is entirely constituted by the so-called unpigmented outer part of it. We have found, extending from the unpigmented part of the cells (the cells proper according to our view) inwards amongst the rods, delicate membranous shreds, and fine true fibres studded with pigment granules.

Hannover[1] has described and figured membranous substances which pass from the six-sided cells and surround the rods. Subdivisions of them appear to surround each rod, and the pigment granules are contained within them. Hannover's figures are more perfect than anything observed by ourselves, but they seem to us only to strengthen the view that these membranes do not belong histologically to the pigment cells, but simply adhere to them, and that they in reality constitute the sheaths to the outer segments of the rods in regard to which there has been so much difference of opinion.[2]

[1] "La Rétine de l'Homme et des Vertébrés." Par Adolphe Hannover. Paris, 1876. The "schemes" intended to illustrate the structure of the retina in Schultze's memoir in Stricker's "Handbook" do not profess to reproduce appearances actually seen. The rapidity with which they have been adopted in English text-books has unfortunately caused this to be lost sight of.

Those who are interested in this difficult and interesting subject should consult Schwalbe's article on the retina in Saemisch and Von Graefe's Handbook, and the above very important memoir by Hannover.

[2] The outer and inner segments of the rods and cones and their pedicles form the ground-substance of that part of the retina which extends from the intergranular layer to the pigment epithelium. The elements of this substance are divided into three strata by the external limiting membrane and a layer of cells between the outer and inner segments. They are covered by investing cells and membranes—the cells having been observed on the pedicles and inner segments and the membranes around the outer segments—and fine fibres, similar to those found amongst the bundles of connective tissue, pass along their surface. They have no special analogies with nerve-structures, and they are not cells. We can only see in them a peculiar modification of the ordinary intercellular or ground-substance, which is connected in some unknown way with the function of vision.

Twin cones are best seen in the retina of fishes. The coloured lenticular body between the outer and inner segments of the cones in birds can be conveniently observed in the hen's retina.

The vitreous humour.—Some of the cells are seen in gold and formic acid preparations, many of them closely resembling lymph cells. It is possible to demonstrate by silver a layer of flat cells on its surface (Ewart), but on account of the feeble consistence of the vitreous humour and the delicate nature of the cells, the operation is one of the most tedious and difficult that can at present be attempted. It is not to be recommended to any one who is not familiar with the application of silver solutions, and who is not prepared to devote much labour to the investigation.

The lens.—Perhaps the most simple method of isolating the lens fibres is one which we have only lately practised. If the lens of a frog is placed fresh in sufficient formic acid (sp. gr. 1020) to cover it, portions may be removed from its surface by needles within a very short period (about half an hour). The portions so removed are teased out in a drop of glycerine, or better in a drop of solution of picric acid, by which they are rapidly stained, and examined in glycerine. The fibres are found isolated, and the characteristic serrated edges are well seen.

If the lens is allowed to remain in the formic acid for 24 hours, it can at the end of that time be entirely broken up, and the fibres stained in formic acid are found isolated in a very perfect condition.

If instead of the undiluted acid, a mixture is used of formic acid (sp. gr. 1020) and water in equal parts, the fibres can be isolated with the same facility and within the same time. In this latter case the lens swells considerably. Having only lately ascertained this property of formic acid, our experiments are not yet sufficiently extensive to enable us to give exact information regarding the effects which may be

found to follow different degrees of dilution or varying periods of maceration.

We have found that the lens fibres of the rat and the sheep are also easily isolable by the same method.

The fibres can also be isolated with rapidity by treatment with the saturated potash solution. After a few minutes a white crust forms on the surface, which can be easily removed and broken up by needles. If the lens is replaced in the solution another separable crust forms, from which the fibres can again be isolated.

If the lens is placed in a sky-blue solution of sulphate of copper (Exner) for 8 to 10 days, sections can be made in which the mosaic of the cut fibres is visible. The fibres can also be isolated in diluted hydrochloric acid (0·1 to 1 per cent.).

The capsule can be seen in fresh, but better in stained preparations (purpurine, aniline). The cells on the posterior surface of the anterior segment of the capsule can be seen in such preparations and also in gold preparations.

Mr. Ewart has demonstrated a layer of cells on the posterior surface of the capsule of the sheep's lens. To show them, cut the eyeball transversely into halves, let the vitreous humour fall out of the anterior half, and pour in gently ½ per cent. silver solution. After some minutes the surface is covered with glycerine and exposed to bright sunlight. As soon as it changes colour it is carefully removed and examined.

Mr. Ewart and ourselves have found that the surfaces of the lens fibres are covered by narrow elongated cells, the nucleus being at one extremity of the cell. These cells are so intimately blended with the surfaces on which they are applied, that their demonstration and isolation are difficult, and only possible by chemical agents. They are most easily seen in the lens of the toad. The lens is removed fresh, and placed immediately in ½ per cent. gold solution, in which it is broken into fragments by needles. The fragments are allowed to remain in the solution for 30 minutes, and are then exposed to sunlight in 2 per cent. acetic acid for several days, or until they acquire a dark colour. They are examined in glycerine. When this method is successful the cell outline is well

marked, and the cell itself has a uniform dark mahogany colour. The nucleus is not distinguishable. The cells are isolable.

If the eyeball of the toad is injected with $\frac{1}{4}$ per cent. gold solution from the aorta, and kept tense for some minutes, the fluid penetrates the lens and occasionally fixes the cells *in situ*. Fragments of the lens are then stained for a few seconds or minutes in concentrated solution of logwood, and examined in glycerine. In these preparations when they are successful, the nuclei of the cells are seen, and the fibres being preserved in their natural condition, the relation of the cells to the fibres can be observed.

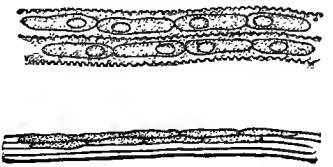

Fig. 13. Lens fibres from the toad. Cells on the flat surfaces and on the borders demonstrated by injection of gold solution from the aorta, and staining with logwood.

Similar preparations can be obtained by injecting from the aorta or carotid of the rat or rabbit.

The cells most frequently observed lie on the broader surfaces of the fibres, and extend nearly from one border to the other. There are other cells which are still more rarely visible. These are of two kinds. In gold injected preparations, very small, narrow, elongated cells are seen applied on the edges of the fibres, their breadth corresponding to the thickness of the fibre. The lens fibre is invested on both its wider surfaces and also laterally by narrow elongated cells.

By breaking up the lens of young rabbits in gold solution, and exposing to light in water strongly acidulated by acetic acid, we found in some preparations layers of rounded cells with large central nuclei. These were easily isolable.

Lens fibres sealed in $\frac{1}{2}$ per cent. gold solution were found after a time to be cut across by dark lines into elongated bars of a fixed and regular length, an appearance which has not yet been explained. It reminded us of the Ranvier constrictions in the medullated nerve fibre.

In potash preparations the nuclei of the lens cells, and very rarely the cells themselves, are visible.

The ear.—To study the membrana tympani, remove it entire with a ring of bone and place it some hours in water. The cuticle can then be removed. It is next placed successively in alcohol, turpentine, and dammar.

For the cochlea and organ of Corti, remove the cochlea from the temporal bone of young animals and decalcify in solution of chromic acid, weak for the first few days and afterwards stronger. When decalcified (8 to 14 days) put it in gum water, harden in alcohol, embed and make sections.

The cochlea must be fresh, and that of the guinea-pig and bat are very suitable. Lavdowsky (Schultze's Archiv., vol. xiii.) recommends osmic acid ($\frac{1}{2}$ to 1 per cent.) and a combination of silver and osmic acid. The object is placed in 1 per cent. silver solution and then for 10 minutes in water, to which a few drops of $\frac{1}{2}$ to 1 per cent. osmic acid has been added. To decalcify the cochlea, he recommends that after it has been hardened in $\frac{1}{2}$ to 1 per cent. osmic acid, it should be placed for a week in Müller's fluid, and then in the mixture proposed by Waldeyer of $\frac{1}{1000}$ per cent. chloride of palladium with a tenth part of hydrochloric acid. It is next saturated with a solution of the purest gum arabic, hardened in common alcohol, and sections are cut. The hardening with gum and alcohol should not be too intense. He recommends as an embedding mass the transparent soap proposed by Flemming, and that the sections should be made without delay and as soon as the soap is cold. Examine in glycerine. Fresh preparations he mounts at once in a mixture of 2 parts glycerine, 2 water, 1 half-concentrated solution of acetate of potash, 1 drop of osmic acid solution being added for each dram of the mixture. For material, young dogs and cats are specially recommended by him.

Fresh preparations should be examined in aqueous humour, and in $\frac{1}{2}$ per cent. salt solution, and nitrate of silver and chloride of gold used *lege artis*.

The cochlea is placed entire in hardening and decalcifying solutions when small, but several openings should be made in

it when large. In most instances this will in any case be required in order to favour the penetration of the solutions.

The membranous semicircular canals are most conveniently studied in cartilaginous fishes such as the skate. The various histological methods can be then easily employed.

Nasal mucous membrane.—The *regio olfactoria* of mammalia is distinguished from the rest of the mucous membrane by its yellowish-brown colour. The epithelium can be examined after maceration in 32 per cent. solution of potash, ½ per cent. solution of chromic acid, 30 per cent. alcohol, or better, ½ to 1 per cent. solution of osmic acid (Exner). The general relations of parts are seen in sections.

The mode of termination of the branches of the olfactory nerve is still unknown, as are also some of the more elementary points regarding the structure of the membrane. It is probable that some of the methods described as applicable to the retina would be found equally useful here, if modified to suit the different anatomical relations.

Organs of taste.—The gustatory buds are found in the papillæ circumvallatæ and papillæ foliatæ. Both systems of papillæ occur in man. Cats have no papillæ foliatæ; guinea-pigs have no circumvallatæ (Frey). The remarks regarding the olfactory mucous membrane are equally applicable here. Careful maceration of the epithelium, sections of the frozen tissue, and staining by solutions of gold and osmic acid are amongst the methods which are to be recommended.

EMBRYOLOGY.

(*The following directions are translated from Dr. Exner's book.*)

The best hardening medium for embryonic tissue is faintly sherry-coloured chromic acid (say ⅛ per cent). Embryos hardened in alcohol are as a rule unsuitable for microscopic examination. A too long action of the chromic acid makes the tissue friable. It is best therefore to harden in chromic acid and then to keep the preparation in alcohol for further use.

The chromic acid is to be occasionally changed, and as the hardening proceeds to be used gradually weaker. An investigation of the first stages of development in which it is necessary to make a continuous series of sections through the entire embryo is attended with great difficulties.

Fishes.—In order to study the first stages of development it is necessary to produce artificial impregnation. The ova of a trout, for example, are received into a large basin of water by gently stroking the belly of the fish. At the same time and in the same way the sperma of the male is obtained and allowed to flow into the basin. The whole is stirred with the plume of a feather.

As soon as the stroking of the female produces an appearance of blood it is to be discontinued.

The fluid is undisturbed for 2 hours. The ova are then removed by means of a horn spoon and a feather, and placed in the apparatus for artificial fish-breeding. This consists essentially in a box, with pebbles on its bottom, half an inch above which a set of glass rods are fixed, parallel to and about two lines from each other.

On these glass rods the ova are so placed that they do not touch each other, and the box is arranged so that a current can pass through it. The current may be so slow that it passes out in drops. It has to be examined daily, and the dead ova, which are recognised by their opacity, removed.

According to the number of successful ova and the length of the hatching process—it lasts 72 days with trout—is to be determined the number which are to be taken out daily. At the beginning they are to be taken out every 12 hours, when the ova should be examined in a watch-glass with a simple microscope in order that the cleavage may be seen. The ova are placed in weak solution of chromic acid, which should be changed as soon as it becomes brown. When they are hard they can be freed from the membrane of the ovum with the greatest ease, and under it the embryo is recognised. It is cut off with part of the yolk, stained, washed in water,

then placed in alcohol, finally in turpentine, then suitably embedded in wax and oil, and sections made gently and slowly with a knife moistened with turpentine. It is advisable to have a knife with a blade hollow enough to retain sufficient turpentine to float the section. Embryos are cut without any lateral movement of the knife, the cutting being done by a pushing more than by a sawing action. At first every section may fall to pieces, embryonic tissue being on account of its friableness very difficult to cut. The sections are immediately floated to the object-glass, the turpentine around them removed with blotting-paper, a drop of dammar varnish added and a cover-glass put on. A paper diaphragm must be placed between the glasses to protect the section. The preparation is immediately labelled, the number of days after impregnation being noted.

Batrachian. The spawn is collected in the pools of marshy places. Toad's ova are scattered through strings of mucus; in the ova of the frog each ovum is surrounded with its own globe of mucus.

Spawn should not be taken unless the ova are quite round, for then only is it freshly laid. The cleavage can be seen in the first hours by the naked eye. Such ova can be preserved as preparations by hardening them in a mixture of equal parts of a 6 per cent. solution of sulphate of copper and 20 to 30 per cent. alcohol. For each ounce of this mixture add one drop of rectified wood vinegar. After 20 hours the membrane of the ovum can be easily removed, and the impregnated ovum preserved in any way that may be desired.

In shallow vessels the fresh ova develop within 6 to 8 days to tadpoles. At the beginning ova should be placed in chromic acid every 6 hours, later once or twice daily. The mucus is removed before they are placed in the fluid.

Embedding and cutting must be carried out with a regard to the following points: The cleavage cavity in the floating ovum is always on the upper surface, and when the ovum is split with a razor from above downwards, the instrument

passes through it. The ova of a later stage should be cut perpendicularly to the semicircular groove which adjoins the vitelline plug of Ecker, now visible on the under surface.

The staining of the sections is on account of the excessive friableness of the batrachian ovum, connected with greater difficulties than is the case with other embryos, but is not so necessary as the elements are sharply defined. The sections cut with turpentine are also brought directly on the object-glass.

Birds.[1]—Impregnated hen's eggs are placed under a sitting hen or are hatched in an incubator, the date being written on the shell. The duration of the period of incubation of the hen's egg is 21 days. The most difficult stages for investigation are again naturally the first hours and days. The embryo floats on the top of the yolk, and can only be removed when the scissors have been passed round it. A horn-spoon or a watch-glass is then useful. In breaking the shell, and in tearing the shell-membrane, it is necessary to be careful not to disturb the embryo which lies close to them. In the first days of incubation the yoke, freed from albumen, is placed entire in chromic acid, and the embryo is not separated from it until after several days, when the vitelline membrane can be removed by gentle agitation of the fluid.

Embryos of the later days are removed from the yolk with a horn-spoon, and after 1 per cent. salt solution has been passed over them, are hardened in chromic acid. Embryos of the first 8 days, on account of their smallness, can be stained entire before being cut. The further treatment is the usual one. Very small embryos can be hardened in osmic acid (until they are faintly coloured), and then preserved entire in dammar varnish.

Mammalia.—It is difficult to procure material for investiga-

[1] Full practical directions for the study of the development of the chick, together with a complete account of the subject, will be found in "The Elements of Embryology," part i., by M. Foster, M.A., M.D., F.R.S., and Francis M. Balfour, B.A.

tion of the first stages of development. In dogs and rabbits used for this purpose, the act of impregnation must be directly observed. In the latter this is so much the easier, as the animals, when they have been separated for some time, pair as soon as they are put together. The female is killed, and the inner surface of the tuba examined with a dissecting lens. When it is spread out on a glass plate and the folds separated by a needle, it is usually easy to find the ova. The ova of the dog are larger, less transparent, and so easier to find. The tuba is fixed on black wax with pins, and carefully split with small scissors. Light is concentrated on the surface with a lens, the ova appearing as white points and generally lying close together.

Embryos of more advanced stages are hardened in chromic acid in the usual way.

INDEX.

THE END.